AROMA AND TASTE IMPACT COMPONENTS IN GRAPEFRUIT JUICE

By

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This thesis is dedicated to Shirdi Sai.

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AROMA AND TASTE IMPACT COMPONENTS IN GRAPEFRUIT JUICE

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This study represents the most comprehensive determination of aroma active volatiles and sulfur compounds in grapefruit juice reported to date.

Initial studies correlated GC peak areas of 52 volatile components (in methylene chloride juice extracts) plus 5 taste components with sensory preference. Highly preferred juices were associated with low myrcene, low linalool and intermediate levels of β-caryophyllene. Since concentrated methylene chloride extracts contained few highly volatile components, a search for a more complete aroma extraction procedure revealed the superiority of pentane-diethyl ether extraction. This extraction gave 73% more top note peak area than methylene chloride liquid-liquid extraction.

Approximately 80 peaks were separated using GC-FID, of which 37-49 components were aroma active. Twenty-five of these aroma active components had intensities high enough to be considered key aroma components. Vanillin was one of the aroma active peaks detected in grapefruit juice for the first time using GC-olfactometry and GC-MS.

A routine method for grapefruit juice sulfur compounds using sulfur chemiluminescence detection was developed for the first time. Twenty-two sulfur compounds were detected. Total peak area increased with pasteurization but decreased with maturity. *p*-menthene-8-thiol, a key aroma impact component, increased as much as 143% after pasteurization.

FID peak areas of 26 aroma active volatile components extracted with pentane-diethyl ether and 5 taste components were correlated with sensory descriptive panel results. Myrcene and α-terpineol correlated negatively with aroma intensity and quality. Grapefruit aroma quality correlated significantly with overall flavor score ($r=0.54$ at $p<0.05$). This is an important conclusion as current industry standards are based only on taste components. The worst juices were effectively separated using taste components whereas aroma active components separated best juices. One hundred percent separation was obtained in the training set when 17 aroma active volatiles and 4 taste components were used to classify the juices based on their quality. This model was tested by evaluating 18 samples not used in the training set. Sixteen of 18 samples were correctly classified within one flavor category. The main application of this flavor model is in

grapefruit juice processing industry where the processors can use it to predict juice quality.

CHAPTER 1
INTRODUCTION

The citrus industry is one of the largest fruit crop industries in the United States. Florida ranks first among the citrus producing regions in North America. Other citrus producing areas include California, Texas, Arizona, and Mexico. The juice produced from citrus fruits also constitutes the majority of fruit juices consumed in the United States and around the world (Kimball, 1991).

Grapefruit (*Citrus paradisi* Mcfadyen) has a highly distinctive flavor with slight bitterness and tanginess. Florida is the world's leading producer of this fruit with a record production of 2.4 million tons in the year 1996-97. However, the value of the crop for this season was $ 68,436,000 which is the lowest since 1969-70 (Citrus Summary, 1997). In Florida, approximately half of the grapefruit grown is processed (Kimball, 1991). A variety of products ranging from pasteurized not-from-concentrate to thermally concentrated frozen grapefruit juice are processed .

Color, taste, and aroma quality of citrus juices can have a pronounced influence on consumer preferences and purchase decisions. According to A.C. Nielsen numbers for supermarkets, the demand for grapefruit juices has decreased (50 million gallons from 1991 to 42.2 million gallons in 1996), while production (61 million boxes of fruits for 1996) of grapefruit increased (Stinson and Barros, 1997). Decreasing popularity of this

fruit is reflected in the economic abandonment of 3 million boxes each of white and colored seedless varieties of grapefruit (Citrus Summary, 1997). Considerable effort has been expended towards the isolation, identification and quantitation of compounds influencing the taste of grapefruit juice (Attaway, 1977; Rouseff et al., 1980; Fellers et al., 1986). The taste factors influencing the flavor of grapefruit juice are sweetness, tartness, balance of sweet/tart and bitterness. The current industry standards also depend on these four factors. Since aroma is a key contributor for any perceived flavor, the purpose of this study was to determine the relative contribution of aroma, and to determine which of these components are important to overall acceptance of grapefruit juice. Specifically, the objectives of this study were to

I. determine the flavor impact components in grapefruit juice, and

II. develop a model that can predict juice acceptance.

These goals can be achieved by

1. Identifying the volatiles in 40 processed NFC Florida grapefruit juices using high resolution capillary gas chromatography and mass spectrometry;

2. Determining the concentrations of bitter compounds in the above juices using high pressure liquid chromatography (HPLC);

3. Determining which volatile components have aroma activity using a gas-chromatography-olfactometry technique (OSME);

4. Evaluating and testing different extraction techniques to determine the technique that produces the most representative volatile profile;

5. Developing an analytical method to determine potent low level sulfur compounds such as 1-*p*-menthene-8-thiol;

6. Training and conducting sensory panels (sniff and descriptive taste panel); and

7. Determining the relationship between sensory and analytical data using multivariate statistics.

CHAPTER 2
LITERATURE REVIEW

Flavor

Citrus juices are becoming increasingly popular due to tbeir unique flavor and perceived health benefits. Flavor is a combination of both taste and aroma. In citrus juices, flavor is affected by taste components like limonin, naringin, sugars and acid, and by volatile aroma compounds. Considerable effort has been expended towards the isolation, identification and quantitation of compounds influencing the taste of grapefruit juice (Attaway, 1977; Rouseff et al., 1980; Fellers et al., 1986). The common consensus of these studies is that a direct relationship exists between bitterness and flavor.

Extensive research has been conducted to identify and quantify volatile components in grapefruit products (Moshonas and Shaw, 1971; Núñez et al., 1985; Cadwallader and Xu, 1994), but few workers have evaluated the relative sensory significance of these compounds. Since 1989, a total of 264 volatile constituents have been reported in grapefruit (Maarse and Visscher, 1989).

Nootkatone was suggested as the key flavor impact compound as early as 1970 (Stevens et al., 1970). However, the importance of nootkatone has been questioned as Shaw and Wilson (1981) found tbat nootkatone when added to oil and juice, had a

significant flavor impact in oil, but very little impact in juice. They concluded that there must be other components that affect the flavor of grapefruit juice. Subsequently, a terpene-thiol, chemically known as 1-*p*-menthene-8-thiol, was reported by Demole et al. (1982), and is now considered one of the most potent flavor compounds found in nature. The authors isolated 7.7 g of dried fraction from 100 L of canned grapefruit juice. A part of this fraction (0.165 g) bad sulfurous odor. There were eight compounds in this fraction. One of the compounds was *p*-menthene-8-thiol, with "a genuine, unmistakable aroma of fresh grapefruit juice". Tbe reported concentration of this compound in grapefruit juice is 0.02 ppb (Maarse and Visscber, 1989), whicb is 200 times its threshold level in the juice (Shaw, 1996). Its aroma threshold in water is $1x10^{-7}$ ppb (Demole et al., 1982). However, until an analytical procedure is developed to quantify this compound at the levels at whicb it exists in juice, it will not be possible to evaluate its relative contribution to grapefruit juice flavor.

Statistical Correlations

Flavor is unquestionably one of the most important attributes of food and is perceived as taste by the tongue and mouth and through the release of the volatile components in the moutb which are sensed retronasally by the olfactory epithelium in the nose (Ohloff, 1990).

Previous workers have developed models based on the correlations between quantified volatile and sensory data (Jennings, 1977; Pino et al., 1986 a, b). A few orange juice volatiles characterized using packed column gas chromatography and non-volatile

components and corresponding sensory hedonic scores were analyzed using multiple regression (Attaway, 1972) or by using principal component analysis (Rouseff and Nagy, 1982). Multivariate statistical programs like principal component analysis (PCA) investigate underlying relationships that exist between variables (Chien and Peppard, 1992). Pino (1982) used linear multiple regression to correlate the sensory and gas chromatography (GC) data of grapefruit juice. Based on correlations, authors selected the variables limonene, α-terpineol, linalool, and myrcene as the most significant in explaining the sensory differences. Velez et al. (1993) classified orange juice samples stored at different temperatures using PCA and GC-analysis. Increased temperature and storage time generally reduced flavor quality. They observed that butanol, α-terpineol and furfural correlated with increasing storage temperatures while linalool and terpin-4-ol correlated best with storage time.

Even though Florida has been a world leader in the production of grapefruit juice, no systematic study to determine the key flavor impact compounds from both aroma and taste has been reported. Using canonical and cluster analysis, Pino et al. (1986a) classified 24 commercial single strength grapefruit juices from different production days and storage conditions. They concluded that nootkatone and an unknown component had positive correlation to flavor while another unidentified component correlated negatively with flavor.

In another experiment, Pino et al. (1986b) correlated sensory and chromatographic measurements of grapefruit juice volatiles using multiple linear regression. Methyl butyrate, ethyl butyrate, limonene, decanal and nootkatone correlated with positive

sensory perception while *trans*- and *cis*-epoxy dihydrolinalool and α-terpineol correlated with unpleasantness of grapefruit juice. The statistical analysis used by the authors identifies those components that change the most with the sensory measurements. In other words, the compounds with high correlations may or may not be aroma active.

Bitterness

Excessive bitterness in grapefruit juice adversely affects flavor and marketability. Compounds that are responsible for bitterness in grapefruit juice are limonin, nomilin, and naringin. These compounds, in moderate quantities, provide the characteristic bite and cleansing of the palette that is liked by most consumers of the juice (Fellers, 1991). However, excessive quantities of these are also detrimental to consumer preference.

Maturity is one of the several factors influencing the content of these bitter components (Berry and Tatum, 1986; Tatum et al., 1972). Albach et al. (1981a) observed that naringin concentration in juice often increased in early spring (February, March, or April) after the onset of rapid vegetative growth. In an other study, Albach et al. (1981b) observed that limonin content was less than 6 ppm by March for most commercial grapefruit varieties. In general, the authors concluded that limonin concentration decreased rapidly as the season progressed, while naringin concentration remained steady until spring, when it began to increase.

Rouseff (1982) reported that nomilin, a limonoid, is twice as bitter as limonin. The authors quantified nomilin and limonin in commercial grapefruit juices produced in the 1978-79 season and observed low nomilin concentrations in all juices. Rouseff et al.

(1980) observed a consistent inverse relationship between bitterness and flavor during a survey of canned single-strength grapefruit juice from 1977-1978 to 1979-1980. They concluded that during a typical season bitterness decreased, flavor increased, limonin decreased and naringin increased with fruit maturity.

Bitterness is one of the 4 basic tastes affecting the quality of juice. Fellers et al. (1987) reported increased bitterness and tartness perception with increasing limonin content, whereas sweetness perception decreased.

Naringin is present in the pulp, rag and albedo of the fruit (Attaway, 1977). The presence of the bitter glycoside naringin in the juice depends upon extraction methods. Therefore, hard squeezing of fruit and excess finishing of juice increases the naringin content in juice.

To meet the requirements of Florida Department of Citrus (Fellers, 1990), blending of different juices is done to keep the limonoids at a moderate concentrations. Various techniques using insoluble polymers, enzymes, and immobilized bacteria (Wilson et al., 1989) have been tried for reducing these compounds in citrus juices.

Immobilized bacterial cells were used by Hasegawa (1983) to reduce the limonin content in orange juice. Carbon dioxide at pressures between 21 and 41 Mpa were used by Kimball (1981) to reduce limonin by 25% from Washington navel orange juice. Ion exchange and adsorbent resins are currently being used to reduce bitter components.

It was reported by Johnson and Chandler (1985) that juice with unacceptably high bitterness can be debittered using IRA-68, S-861, and IRC-84 resin columns to produce an acceptable Florida grapefruit juice. Residence time in the column bed and the

temperature of the bed was found to be critical in reducing the amount of limonin and naringin (Wilson et al., 1989).

GC-Olfactometry

A hybrid technique has recently been developed that directly measures only those components that are causative (that is, have aroma activity). It combines the resolving power of a capillary gas chromatograph with modern sensory analysis. The technique is called gas-chromatograpby olfactometry (GC-O). It utilizes a human assessor to determine which of the many chromatographic peaks have aroma activity and characterizes that odor. Some of the GC-O techniques available today are Charm® Analysis, Aroma Extraction Dilution Analysis (AEDA) and OSME which is a time intensity method. Charm® Analysis and AEDA are based on the determination of odor detection thresholds of the compounds through a series of dilutions while OSME determines intensities without dilutions.

Charm® Analysis

Acree et al.(1984) developed the Charm® analysis technique, and has used this technique to evaluate a variety of products. Cunnigham et al. (1986) analyzed apple volatiles and identified tbe 12 most odor active peaks. A generalized description of apple odor produced by combining samples showed beta-damascenone, butyl, isoamyl, and hexyl hexanoates, along with ethyl, propyl and hexyl butanoates, to be important to tbe odor of most apple cultivars. Differences between fresh and pasteurized orange juices

were characterized by Marin et al. (1992) using this technique. The authors observed large changes in odor activity for linalool, ethylbutyrate, vanillin and several unknown components.

AEDA

Aroma extraction dilution analysis, developed by Schieberle and Grosch (1984), is based on serial dilutions like the Charm® analysis. In this method, serial dilutions (1:2) are made and analyzed until the odor is perceived by human subjects. The resultant intensities are plotted in an aromagram. Schieberle and Grosch (1988) used AEDA to identify indicator substances for the assessment of the deterioration of lemon oil flavorings in acidic foods. Fresh samples and samples stored for 30 days (at 37°C) were compared. The study suggested that *p*-methyl acetophenone, *p*-cresol, *p*-cymene, and fenchyl alcohol are the most potent storage indicator components in the lemon oil.

Hinterholzer and Schieberle (1998) identified the most odor active volatiles in hand squeezed juice of late Valencia oranges. The authors identified ethyl butyrate (fruity), *Z*-hex-3-enal (green) and 3,4,5,7-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (sweet, spicy) as the potent odorants with highest flavor dilution factor.

OSME

da Silva et al. (1994) claimed that the dilution techniques mentioned above would not give accurate information, since the odorants have different intensity functions above

their threshold levels. The authors proposed and developed a new GC-O methodology based on psycho-physical laws called OSME (Greek word meaning smell).

OSME is a time intensity procedure which determines the intensity of the perceived odor without dilution. In this method, the trained subjects sniff the effluents from GC mixed with humidified air, and directly records the odor intensity and duration of each odor active component while describing its odor quality. Intensities of individual components are plotted versus elution time and the resultant graphical representation is known as an aromagram.

Orange aqueous essence was analyzed by Bazemore (1995) using OSME. Octanal, linalool, and ethyl butanoate were found to have the strongest aroma in both reflux and no reflux samples of aqueous orange essence.

OSME has also been used to differentiate Pinot Noir wines from grapes of different maturities (Miranda-Lopez et al., 1992). Spicy (ethyl octanoate), vegetative, herbal, and vanilla (ethyl vanillin) aroma's were detected in wines made from late maturity grapes. The authors also found that 45 to 60% of odor active peaks found in GC-O were not detected by an analytical detector (GC-FID).

One characteristic feature of GC-O methods is the occurrence of peaks in the aromagram which might not match a corresponding FID peak. This occurs because the human nose is much more sensitive to some of the compounds than are analytical detectors. Mistry et al. (1997) detected a musty off-flavor in the extracts of beetsugar. However, no FID peaks were detected in the region that produced the most aroma

activity. Upon enrichment of the extract by the authors, geosmin was identified as the compound producing the musty odor.

Sample Preparation

Extraction and isolation of the representative aroma compounds in a food matrix is one of the critical steps in flavor research. No single extraction method can be considered universal, rather the extraction procedure employed depends on the needs of the researcher and the nature of the sample. Various isolation procedures for volatile components have been compared by many researchers. Weurman (1969) presented an in-depth description of different isolation techniques used in odor research. In this study, several different extraction techniques were evaluated for optimum odor recovery.

Nunez et al. (1984) compared five methods including solvent extraction (batch wise and continuous), distillation and simultaneous distillation solvent extraction-SDE, (Likens-Nickerson and Godefroot et al. apparatus) for volatile components of grapefruit juice. The two SDE methods were reported to be most suitable for grapefruit juice in terms of rapidity, reduced solvent removal and strong representative odor of the sample.

Jennings (1977) sampled peach volatiles with the Likens-Nickerson apparatus and porous polymer traps. The polymer trap essence exhibited larger amounts of lower boiling compounds than did the distillation extraction essence. When extended trapping periods were utilized, higher boiling compounds were also present in the polymer trap essence extract.

Moshonas and Shaw (1971 and 1982) isolated orange juice volatiles using dichloromethane solvent extraction. Ethanol was not extracted by this method, which aided in the analysis of other compounds normally masked by the large ethanol peak.

Moshanas and Shaw (1992) compared the static and dynamic head space metbods for orange juice volatiles. Acetaldehyde, methanol, methyl butyrate, α-pinene, γ-terpinene decanal and linalool were extracted in greater quantities by static bead space, while etbyl butyrate, hexanal, etbyl bexanoate and cis -3-hexenol were higher in dynamic head space.

Umano and Shibamoto (1988) described a new method in whicb head space volatiles were purged into water in a gas washing bottle and simultaneously continuously extracted with dichloromethane. An aqueous solution containing (cysteamine) was used to trap aldehydes (as derivatives of thiazolidine) and a phenylenediamine solution to trap dicarbonyls (as quinozalines). GC revealed 22, 25 and 130 peaks in the whole grapefruit, grapefruit juice and grapefruit peel extracts respectively, the predominant component being limonene in all cases.

SPME

Solid-pbase micro extraction (SPME) is a relatively new technique in which analytes of interest partition from the sample matrix into a polymeric solid coating. SPME was first reported by Zbang et al. (1994) and has been used in qualitative and quantitative studies of citrus juices (Matich et al., 1996).

Comparisons between traditional bead space Tenax adsorption/desorption and head space SPME were made by Pelusio et al.(1995). According to the author, when

polydimethylsiloxane fiber coating was used, GC-MS analyses of the aromas showed that the SPME technique was less suitable for quantitative analysis due to lower affinity of the fiber for more polar and very volatile compounds.

Steffen and Pawliszyn (1996) reported 1- 20% relative standard deviation for most components in orange and grapefruit juices analyzed by SPME. According to Xiaogen and Peppard (1994), addition of salt enhanced the amount of volatiles absorbed using SPME.

SPME GC-MS enabled detection of more than 50 volatile compounds including hydrocarbons, aldehydes, carboxylic acids, phenolic compounds, esters, ketones, lactones, alcohols, N-containing compounds and S-containing compounds in the head space of milk powder (Stevenson and Chen, 1996). Chin et al. (1996) observed that SPME fibers extracted major cheese volatile components, but minor components such as volatile sulfur compounds were not observed.

The principle behind SPME is the partitioning of analytes between sample matrix and the extraction medium (Zhang et al., 1994). The amount absorbed by the coating at equilibrium is directly related to the concentration of the component in the sample $n = K_{fs}V_fC_0V_s / (K_{fs}V_f+V_s)$ where n is the mass of the analyte absorbed by the coating; V_f and V_s are volumes of coating and sample respectively; K_{fs} is the partition coefficient of the analyte between the coating and the sample matrix; C_0 is the initial concentration of the analyte in the sample. However, since $V_s >> K_{fs}V_f$, in food analysis, the earlier equation can be simplified as $n = K_{fs}V_fC_0$ and hence is independent of the sample volume. This is one feature that makes SPME suitable for food analysis.

Sulfur Compounds

Sulfur compounds play a major role in determining the flavor characteristics of many food substances. Sulfur compounds are often formed as a result of the enzymatic process when plants are cut or chewed, releasing flavor precursors and enzymes from rupturing cells. Sulfur components are unusual since in low concentrations they are responsible for many positive sensory qualities in foods and flavorings. However, higher levels of the identical compound often result in off flavors (Tressl and Silwar, 1981). The authors reported that furfurylmercaptan at 10-500 ng/L had a fresh roasted coffee aroma, while at 1000 ng/L a sulfury stale coffee aroma was perceived.

Another aspect of organic sulfur compounds at low concentration is the influence of functional groups (Boelens et al., 1993). The authors reported that the odor threshold values of tertiary thiols are 300 - 3000 times lower than those of primary and secondary thiols. The example they quoted for beer is 2-methyl-2-propanethiol which has a threshold value of 80 units, while 2-methyl-1-propanethiol has a value of 2500 units. Although, sulfur components are present only in trace quantities in most food materials, their contribution to the overall flavor quality is significant due to their extremely low aroma thresholds.

In spite of the significant role of sulfur compounds in the food matrix, there are only a few reports regarding their affect in citrus juices. Shaw et al. (1980) detected hydrogen sulfide, methyl sulfide, sulfur dioxide, methane thiol, and some higher alkyl

sulfides using a flame photometric detector in orange juice samples. Since concentrations of H_2S and methyl sulfide in orange juice were greater than their reported aroma thresholds, these components may have a significant impact on overall juice quality. In another study, Shaw and Nagy (1981) concluded that early season orange and grapefruit juice had higher levels of H_2S. When sensory analysis was conducted on these juices, the panelists reported a harsher (pungent) aroma, and the authors attrihuted this to higher H_2S levels. This attrihute was not detected by the authors in late season orange and grapefruit juices.

Demole et al. (1982) isolated and characterized *p*-menthene-8-thiol, which had the "unmistakahle aroma of fresh grapefruit." They found that when comhined with nootkatone, the mixture gave a "full hodied flavor" of fresh grapefruit. *p*-menthene-8-thiol undergoes cyclization to form 2,8,-epithio *cis-p*-menthane, which also has a characteristic grapefruit aroma. The odor threshold of this compound was 9 ppb (Maarse and Visscher, 1989). The cyclization reaction takes place at room temperature in the presence of light and these two compounds are reported to co-occur in grapefruit (Demole et al., 1982).

CHAPTER 3
MATERIALS AND METHODS

A major goal of this project was to quantify and characterize the aroma impact components in not-from-concentrate grapefruit juices. Non-volatile flavor attributes such as sweetness, sourness and bitterness were also evaluated by measuring °Brix, titratable acid, limonin and naringin separately in order to evaluate the relative contribution of taste vs. aroma components. Sensory attributes were quantified and correlated with analytical measurements. Experimental design and analytical techniques used to achieve this objective are discussed in this chapter.

Grapefruit Juice Sample Collection

Survey Samples

Methylene chloride extracts

Twenty-nine not-from-concentrate (NFC) grapefruit juice samples were obtained from processors with processing dates ranging from November, 1995, to June, 1996, and stored at -8 °C until analyzed. Both red/pink and white juices were used in this study. Authentic solvents were purchased from Fisher Scientific (Pittsburgh, PA). Standards used for quantifying volatiles and non-volatiles were purchased from Aldrich Chemical

Company Inc. (Milwaukee, WI). A few standards were obtained as gifts from SunPure, Inc. (Lakeland, FL) or Givaudan Roure (Lakeland, FL).

Pentane-diethyl ether extracts

Forty not-from-concentrate (NFC) grapefruit juice samples (2 QT gable top cartons) were purchased from a local supermarket with manufacturing dates ranging from January, 1997, to June, 1997, and stored at -8 °C until analyzed. Both red/pink and white juices were used in this study. Sources for solvents and standards were the same as for the methylene chloride extracts study.

GC-Olfactometry Samples

Early (November, 1996), mid (January, 1997) and late (May, 1997) season grapefruit juice samples were obtained from the Florida Department of Citrus. Grove run red grapefruit were purchased from a local packinghouse and processed in the pilot plant located at the Citrus Research and Education Center, in Lake Alfred. Fruits were washed, dried and sized for the extractors in the pilot plant. Extraction was accomplished using commercial FMC model 391-B and 491 extractors with standard juice settings. An FMC model 35 juice finisher was used with a moderate squeeze setting. The finished juice was pumped to the holding tank prior to pasteurization. Pasteurization was done using a Feldmeier tube-in-shell pasteurizer. The juice was heated to 90.6°C at a flow rate of 1 gallon per minute. Samples were packaged in 32 oz clear glass bottles and stored at -8 °C

until analyzed. Both unpasteurized and pasteurized red grapefruit juices were obtained. Samples consisted of two bottles for each juice type.

Sample Preparation

Liquid-Liquid Extraction with Methylene Chloride

Extraction of volatiles was accomplished with methylene chloride using the method described by Parliament (1986) and modified by Klim and Nagy (1992). Eight mL of juice were added to 4 mL of methylene chloride and mixed using a Mixxor-like apparatus. The apparatus consisted of two syringes : 50 cc and 30 cc capacity connected with an 8 cm long, 3 mm outer diameter stainless steel connector. The mixture of juice and solvent was poured in the larger syringe and, using forward and backward motion, the mixture was pumped into and out of the smaller syringe. The juice and the solvent were mixed for ca 2 minutes. The emulsion was broken by centrifuging for 10 min (15000 g). The lower solvent layer of approximately 3 mL was collected for analysis. An internal standard, 6 µL of propyl benzene, was added and the extract was concentrated to about 30 µL in a 100 µL graduated taper vial. Concentration was accomplished by blowing nitrogen gas at a flow rate of 40 mL / min across the surface. Concentrated extracts were prepared fresh every morning and analyzed the same day. Each juice sample was extracted twice and each extract analyzed in duplicate.

Liquid-Liquid Extraction with Pentane-Diethyl ether (1:1):

Extraction of the volatiles was accomplished according to the previously described method except a 1:1 mixture of pentane and diethyl ether was used in the place of methylene chloride. Two internal standards, propyl benzene (50 µL of 100 ppm) and 2-heptadecanone (25 µL of 500 ppm), were added to 8 mL of juice and extracted. The extracts were concentrated to 50 µL using the same procedure as that previously described. Each sample was analyzed in duplicate.

Dynamic Head Space Purge and Trap Solvent Elution

Dynamic head space extraction was accomplished using a two necked 25 mL round bottom flask. Ten mL of juice were added to the flask along with a stir bar. Nitrogen was impringed upon the juice surface at a rate of 40 mL / min through one of the flask necks. To the other opening, a 2 mm i.d. glass column comprising powdered charcoal (Supelco, Bellefonte, PA) and Tenax® (Supelco, Bellefonte, PA) in a 1:3 (v/v) ratio was attached. Juice was heated to 37 °C using a constant temperature water bath. Volatiles were trapped in the column for 30 min. The column was removed and purged with dry nitrogen (20 mL/min) for ca.1 minute to reduce trapped moisture.

Three mL of (1:1) pentane and diethyl ether mixture were used to elute volatiles from trap materials. Extracts were concentrated in the same manner as with the methylene chloride extracts. The column was cleaned both before and after extraction using 3 to 4 times the column volume of pentane.

Extraction Procedure for Sulfur Compounds

Extraction of the volatiles was accomplished using the same method as that described for methylene chloride except ethyl acetate was used as the solvent. S-methyl thio butanoate (15 μL of 10 ppm) was added to 8 mL of juice as an internal standard and extracted. Extracts were concentrated to 50 μL using nitrogen with the procedure described earlier for the methylene chloride extracts. All samples were analyzed in duplicate.

Extraction Procedure for GC-Olfactometry Analysis

Extraction of juice volatiles was accomplished using the method described for pentane-diethyl ether extractions. Two internal standards, benzaldehyde (25 μL of 5000 ppm) and methyl jasmonate (25 μL of 5000 ppm), were added to 8 mL of juice and extracted. Extracts were concentrated to 50 μL using dry nitrogen as previously described. Each sample was analyzed four times using three detectors (Flame Ionization Detector (FID), Sulfur Chemiluminescence Detector (SCD) and OSME).

Instrumental Techniques

GC-Flame Ionization Detector

Individual volatile constituents were separated using an HP-5890 GC (Palo Alto, CA) with a flame ionization detector and a 30 m x 0.25 mm i.d. x 0.5 μm film thickness

low bleed DB-5 column (J&W Scientific; Folsom, CA). The oven temperature was programmed from 35 to 275 °C at 6 °C/min with helium at a flow rate of 2.19 mL/min (34.6 cm/sec linear velocity). The injector temperature was maintained at 250 °C and detector temperature at 320 °C. The nitrogen gas flow was maintained at 19 mL/min, while air and hydrogen flows were maintained at 296 and 35 mL/min, respectively. The injection volume was 1 μL for methylene chloride extracts and 0.5 μL for pentane-diethyl ether and ethyl acetate extracts. Injection was split-less. Chromatograms were recorded and integrated using Chrom Perfect (Justice Innovations, Mountain View, CA). The data acquisition rate was 10 pt/sec. Chromatograms for methylene chloride extracts were recorded and integrated using an APEX Chromotography Workstation (Autochrom Inc., Milford, MA) with a four channel data system. Data acquisition rate was 0.4 s/point.

GC-Sulfur Chemiluminescence Detector

Volatile constituents were separated using an HP-5890 GC (Palo Alto, CA) equipped with a sulfur chemiluminescence detector (Seivers Instruments Inc., Boulder, CO) and a 30 m x 0.25 mm i.d. x 0.5 μm film thickness low bleed DB-5 column (J&W Scientific; Folsom, CA). Oven temperature was programmed from 35 to 275 °C at 6 °C/min with helium at a flow rate of 2.19 mL/min. Injector temperature was maintained at 250 °C. Internal temperature of the SCD burner head was 780 °C. Air and hydrogen were maintained at 114 and 9 mL/min respectively. Cell pressure was maintained at 5.5 torr and the ozone at 8.75 psi. The injection volume was 0.5 μL in split-less mode.

Chromatograms were recorded and integrated using Chrom Perfect (Justice Innovations, Mountain View, CA). The data acquisition rate was 10 pt/sec.

GC-OSME

The individual volatile constituents were separated using an HP-5890 GC (Palo Alto, CA) with a sniff port (DATU, Geneva, New York) and a 30 m x 0.25 mm i.d. x 0.5 µm film thickness low bleed DB-5 column (J&W Scientific; Folsom, CA), with helium at a flow rate of 1.55 mL/min. Oven temperature was programmed from 35 to 275 °C at 6 °C/min Injector temperature was maintained at 250 °C and detector temperature at 320 °C.

Purified air was obtained by passing compressed air through drierite and a molecular sieve 5A (Alltech, Deerfield, IL) and directed into a temperature controlled, water filled round bottomed flask fitted with fritted glass impringers. Water temperature was maintained at 35 °C. Airflow through the sniff port was 11.2 L/min. The stainless steel sniff port tube was 70 cm long and 1 cm in diameter.

Sniffing began after the solvent had eluted off the column (ca 3 minutes). Panelists were requested to sit in a comfortable position and asked to indicate their responses using a linear potentiometer (variable resistor). The device had a pointer which the subject moved from left to right and back again across a 15 point structured scale (0=none, 7.5 = moderate and 15 = extreme). Time and intensity were recorded by the OSME soft ware system, installed on a 386-PC. The component odor was described by the panelist and recorded by the researcher. Maximum sniffing time was 30 minutes.

GC-Mass Spectrometry

All GC-MS data were collected using a Finnigan GCQ Plus system (Finnigan Corp, San Jose, CA) using helium (99.999%) for the GC carrier gas and the collision/bath gas in the ion trap. Injector temperature was 250 °C. Samples (0.2-1.0 µL) were injected using the split less mode with a purge time of 1.5 min. The initial column temperature was beld at 35 °C for 3 min followed by a 4 °C/min temperature ramp to 221 °C which was followed by a 10 °C/min ramp to 275 °C which was held for 1.1 min to elute high boiling components in extracts. Linear velocity was 31.9 cm/sec through a 30 m x 0.25 mm id, 0.25 m RTX5-MS column (Restek Corp, Bellefonte, PA). Transfer line and ion source temperatures were 275 °C and 170 °C. The mass spectrometer had a delay of 4 minutes to avoid the solvent peak, and then scanned from m/z 40 to m/z 300 in order to achieve 7 spectra per second. Ionization energy was set at 70 eV.

Limonin and Naringin Analysis Using HPLC

Sample preparation

Limonin and naringin for grapefruit juice samples (extracted with methylene chloride) were analyzed according to the method developed by Widmer and Martin (1994). In a 10 mL volumetric flask, 5 mL of juice were equilibrated for 5 min at 90 °C. The sample was diluted to 10 mL with 40 % acetonitrile and filtered through a Whatman GDX 0.45 µ filter. About 2 mL of filtered sample were placed into 2.5 mL Snap-Its™ (National Scientific Company, Quakertown, PA) glass vials and used for further analysis.

For grapefruit juice samples extracted with pentane-dietbyl ether solvent mixture, limonin and naringin analysis was similar to the procedure described above, except that these were not heated prior to HPLC analysis.

HPLC instrumentation

A Thermo Separations (San Jose, CA) LC system (Spectra Focus Optical Scanning detector and P4000 gradient pump) with a Spectra Physics AS 3000 (San Jose, CA) auto sampler was used for the analysis of limonin. A Waters 6000A pump (Milford, MA) with a Waters 440 (Milford, MA) two channel UV absorbance detector equipped witb a 280 nm filter was used to determine naringin. Chromatograms were recorded and integrated with a Thermo Separations 4290 (San Jose, CA) integrator and Winner on Windows 4290 (San Jose, CA). Separations were achieved using a 4.6 mm x 150 mm 5 μ CN analytical column (MacMod Analytical Inc., Chadds Ford, PA) for limonin and a 4.6 mm x 150 mm 5 μ C-18 analytical column (Kromasil C-18, Higgins Analytical, Mountain View, CA) for naringin. The mobile phase consisted of water /acetonitrile (80.5:19.5) for naringin analysis, and water / acetonitrile (63:37) for limonin analysis. The injection volume was 40 μL and flow rates of 1.0 mL/min were used.

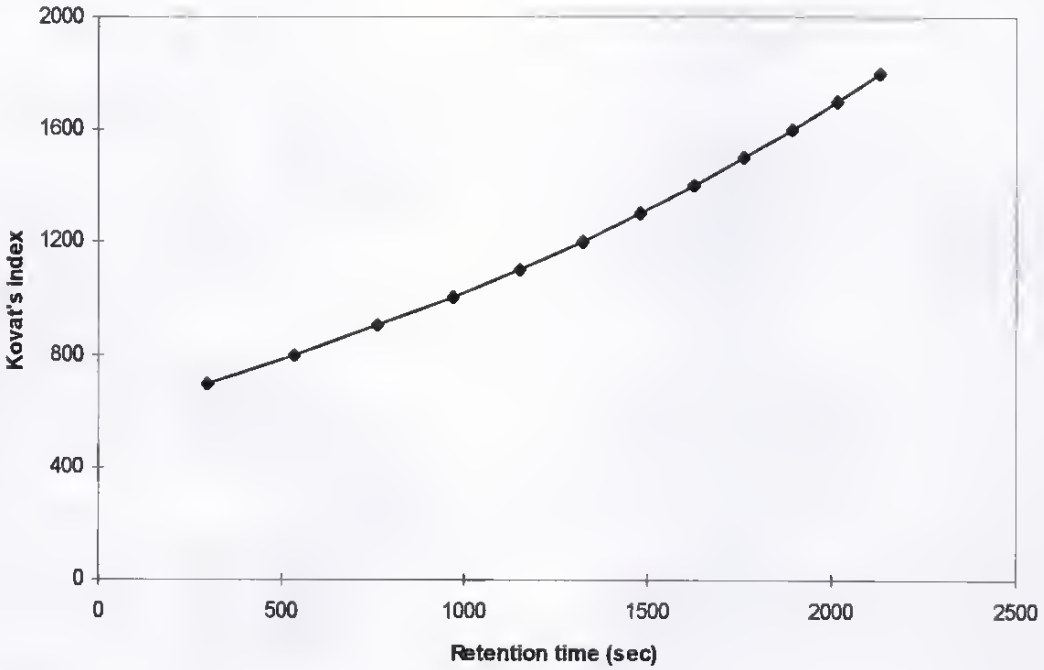
Peak Identification and Quantification

Chromatographic peaks were identified using their mass spectra and comparison of tbeir observed Kovat's index with published Kovat's retention indices (Kovats, 1965).

Calculation of retention indices for individual peaks was done using retention time data from a series of alkane standards run under the same conditions. Alkane standards (Supelco Inc. Bellefonte, PA) from C 6 to C18 were used for this. Kovat's Indices for these standards were calculated by multiplying the corresponding carbon number by a factor of 100. Retention time (seconds) for the standards were plotted against their corresponding Kovat's Indices (Figure 1). The resulting plot was used to fit an equation, which was then used to calculate the retention indices for individual grapefruit juice volatile components.

Quantification of some of the GC-FID peaks from early, mid and late season grapefruit juice was done by using authentic standards obtained from Sun Pure, Inc. (Lakeland, FL). Solutions of ethylbutyrate, propyl benzene, sabinene, myrcene, octanal, linalool, decanal, nerol, β-caryophyllene, nootkatone, 2-heptadecanone were prepared at concentrations ranging from 22 to 227 ppm and injected in duplicate. Calibrations plots were generated by plotting the peak areas versus sample concentration. Sample plots generated for 4 components are shown in Figure 2. Equations for the rest are given in Table 1. FID peak areas obtained for grapefruit juices were normalized using the peak area of internal standard.

Quantification of peaks from GC-SCD was done by analyzing s-methyl thiobutanaote at five concentrations (10, 5, 1, 0.01, 0.001 ppm) in duplicate. The calibration curve for these is shown in Figure 3.



$KI = a + (b*t^2 + c\sqrt{t} + d) * \ln(t)$

Where KI - Kovat's retention index

t - Retention time in seconds

and constants

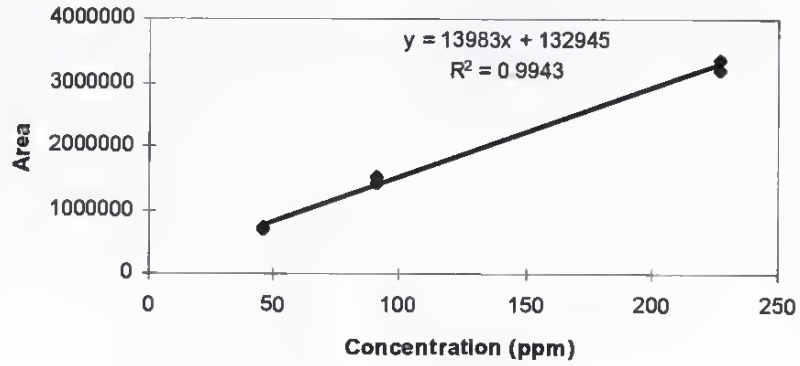
a = 592.525

b = 2.003e-5

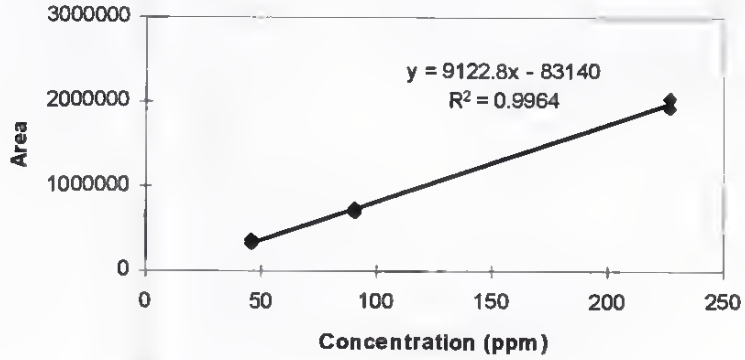
c = 1.703 and

d = -12.342

Figure 1. Standard curve used for calculation of kovat's retention indices for volatile components.



(A)



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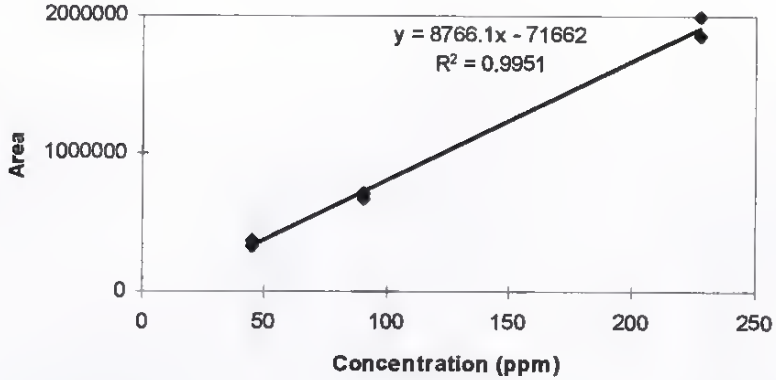
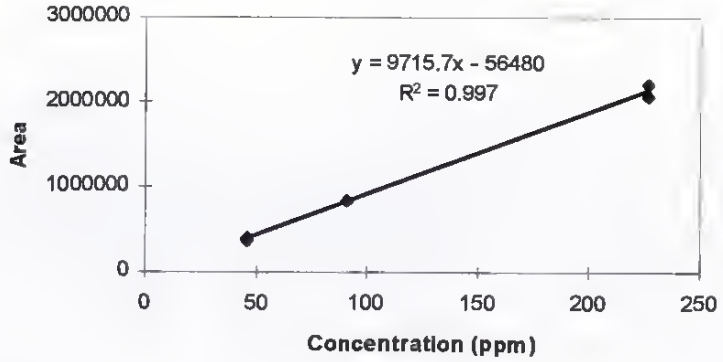


(C)



(D)

Figure 2. Calibration curves used for quantifying the volatiles. (A) propyl benzene, (B) myrcene, (C) linalool, (D) nootkatone.

Table 1. Calibration equations used for calculating the concentrations of components detected in GC-FID.

Component	Linear regression equation	r-squared
Ethyl butyrate	6128*x - 29184	0.997
Propyl benzene	13983*x + 132945	0.994
Sabinene	7586*x - 61320	0.997
Myrcene	9123*x - 83140	0.996
Ocatanal	9093*x - 108034	0.968
Linalool	8766*x - 71662	0.995
Decanal	4951*x - 44666	0.999
Nerol	9178*x - 69883	0.990
Caryophellene	8257*x - 80289	0.993
Nootkatone	9716*x - 56480	0.997
2-heptadecanone	13889*x - 16990	0.994

Note: x in the linear regression equation represents the area of the peak to be quantified

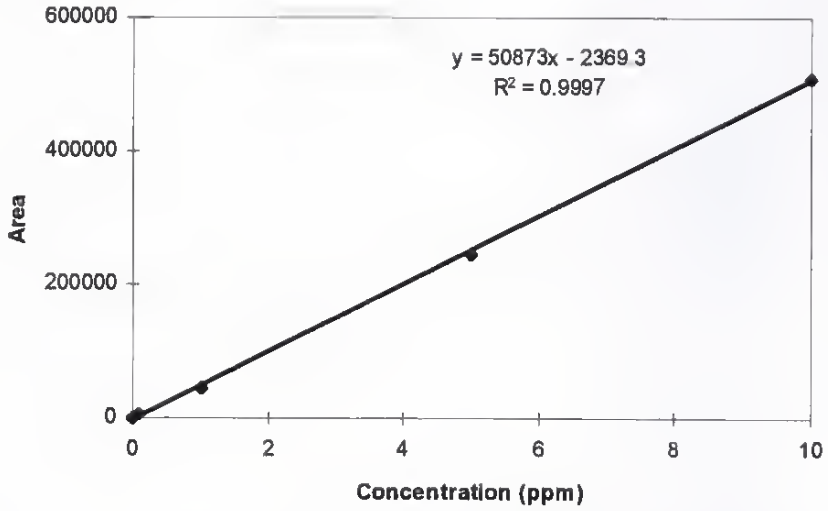


Figure 3. Calibration curve for s-methyl-thiobutanoate (sulfur compounds).

Sensory Analysis

DOC Preference Panel

Bitterness taste thresholds of individual taste panelists were determined using 5-50 ppm of limonin and 150-950 ppm of naringin aqueous solutions. Twenty-four untrained panelists were used. A nine-point hedonic scale (forced choice) was used with 0 indicating dislike extremely, 9 indicating like extremely and 5 indicating neither like nor dislike. Panelists were presented with three samples under illumination with red light and asked to rate their preference. Samples were coded with random three digit numbers randomly arranged on serving trays, and then presented to panelists.

USDA Descriptive Panel

This panel consisted of 12 trained panelists. Taste threshold characteristics of individual taste panelists were determined using 5-50 ppm of limonin and 150-500 ppm of naringin solutions. The attributes rated were grapefruit aroma intensity, grapefruit aroma quality, bitterness, balance of sweetness/tartness and overall flavor quality. A 15 cm line segment scale was used with 0 indicating least intensity, 15 indicating highest intensity. A sample ballot given to the panelists is represented in Figure 4. Panelists were presented with four samples and a reference juice.

The reference juice (10 gallons: pasteurized, not-from-concentrate) was obtained from a local juice processor and stored in 2 L amber colored glass bottles at -8 °C.

Grapefruit Juice Sensory Panel

Please Read the Instructions

Name: **Sample Number**: 321
Date:

Aroma Analysis: Uncover the sample, take a deep sniff, and rate the quality & intensity for the grapefruity aroma.
Taste the juice and mark down the intensity for Bitterness and Sweetness.
Based on the above attributes rate the **overall flavor quality** of the juice

Grapefruit Aroma Intensity

0 — 15
None — Strong

Grapefruit Aroma Quality

0 — 15
V.Poor — V.Good

Sweet/Tart Balance

7 — 0 — 7
More Sour Than Sweet — More Sweet Than Sour

Bitterness

0 — 15
None — Strong

Overall Flavor Quality

0 — 15
V.Poor — V.Good

Comments: (If any **Off Flavor** is percieved describe the attribute and rate it as **None, Moderate or Strong**.
Any additional comments are also welcome).

Figure 4. Sample ballot for the grapefruit juice descriptive sensory panel.

Panelists were given this juice 6 times over 3 weeks and the scores for individual attributes were averaged. Average scores for all attributes were marked on the ballot sheet to serve as reference points for other samples. Panelist's consistency was checked by giving the reference sample after every 10 grapefruit juice samples.

Samples were coded with random three digit numbers randomly arranged on serving trays, and then presented to panelists.

Training of Panelists

GC-Olfactometry Panel

The panel consisted of 2 males and 1 female. Training consisted of three practice runs with grapefruit juice extracts to familiarize the panelists with the sliding scale, optimum positioning and breathing technique, and to provide practice with verbal descriptors. In addition, a mixture of standard components typically found in grapefruit juice was injected to familiarize the panelists with these odors and to help standardize their descriptors. The results from the standard mixture are presented in Results and Discussion section. To condition the olfactory senses, individual standard solutions (20 mL at concentration of 4 ppm) were smelled by the panelists prior to OSME analysis of all the grapefruit samples. The standards consisted of hexanal, ethyl butyrate, myrcene, linalool, decanal, α-terpineol, *p*-menthene-8-thiol, and nootkatone.

Descriptive Panel

Twelve members (6 women and 6 men) were recruited from the United States Department of Agriculture, Winter Haven, FL for a descriptive taste panel. The panelists were of varied age groups and ethnic backgrounds. All panelists had some prior citrus taste panel experience. Minimum and maximum values for ratio of total soluble solids : % acid from the United States grapefruit juice grading system were used to train panelists. Brix:acid ratio of 14 : 1 (70 g of sucrose and 5 g of citric acid) and 8 : 1 (40 g of sucrose and 5 g of citric acid) were prepared using food grade sucrose and citric acid in water. Naringin solutions of 200, 100 and 25 ppm (in water) were used for a bitterness standard. All solutions were prepared using double distilled water. Fresh squeezed grapefruit juice and fresh grapefruit peel were used as standards for grapefruit aroma quality and intensity.

Statistical Analysis

Principal components analysis in SAS (Version 6.11, SAS Institute, Cary, NC) was used to evaluate the data set from the preference sensory panel and GC-FID data. Univariate statistics and step wise multiple regression (forward) with Wilks Lambda was also employed to identify those components which would be most differentiating between sensory classifications. Canonical discriminant analysis (STATISTICA version 5.0, Stat Soft, Tulsa, OK) was used to identify the peaks which would help in differentiating the juice preference groups. The cross-validation component in this section was employed to determine the classification significance for each sample. Mahalonobis distances were

used to judge the distances between the juice groups. Posterior probabilities were used to predict the juice quality.

CHAPTER 4
RESULTS AND DISCUSSION

Correlations Between Preference and Analytical Measurements

Initial attempts to determine the aroma impact components of grapefruit juice utilized methylene chloride extracts. Methylene chloride extraction was chosen as a means of extracting aroma volatiles as it had been used as a solvent by several authors for isolating citrus volatiles (Moshanas and Shaw, 1971; Parliament, 1986; Klim and Nagy, 1992). Figure 5 represents a typical chromatogram from a grapefruit juice methylene chloride extract. It is important to note the relative absence of early eluting (low boiling) components. Over 125 chromatographic peaks were resolved in the chromatogram. However, some peaks were too small to be accurately quantified. Of the original 125 peaks in the chromatogram, 52 were selected for further studies. Identification of these peaks was based on Kovat's retention index values and mass spectral data.

Maximum, minimum and average area values for these peaks are given in Table 2. All components identified in Table 2 were also identified by Núñez et al. (1985) and Maarse and Visscher (1989).

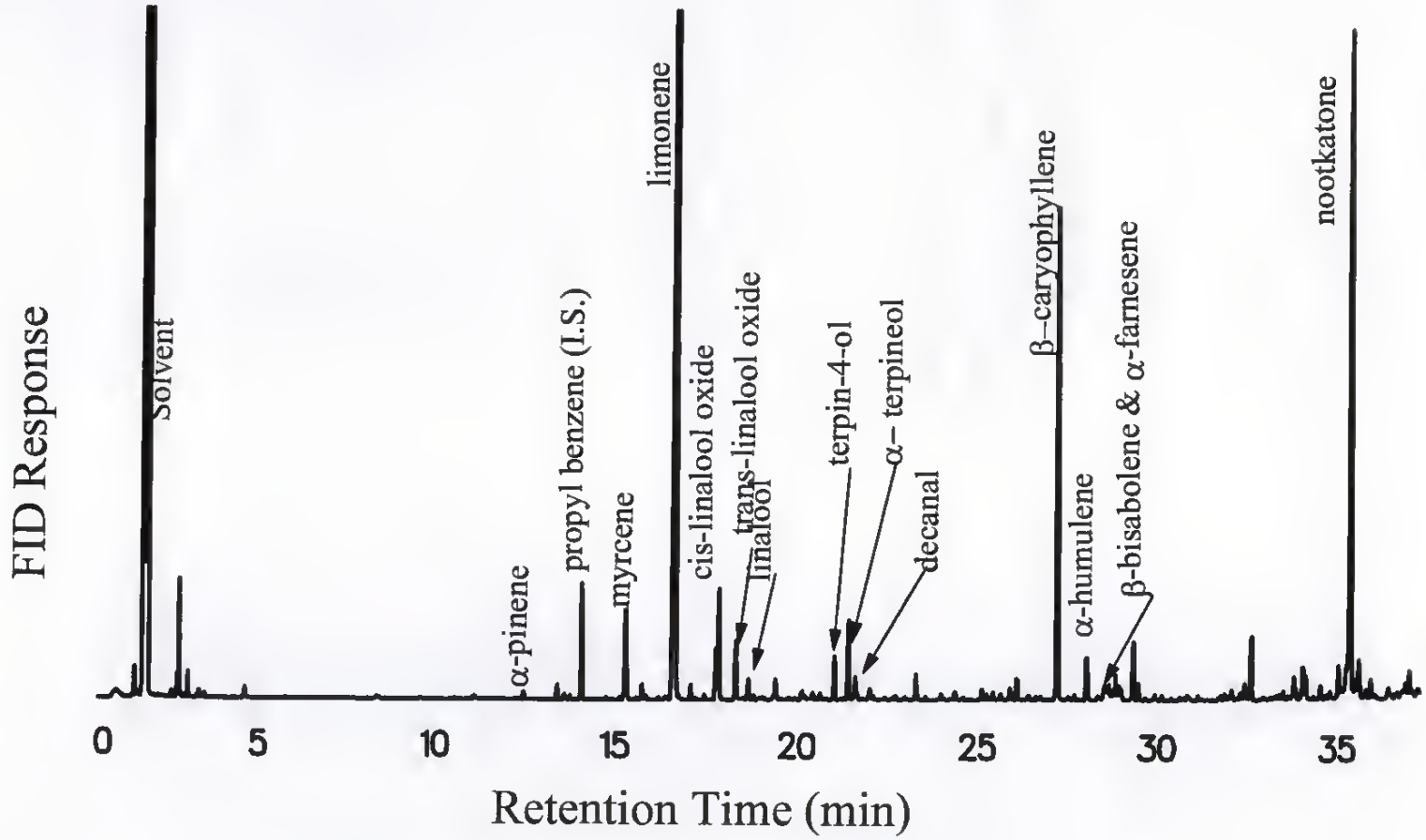


Figure 5. Chromatogram of not-from-concentrate grapefruit juice methylene chloride extract.

Table 2. Maximum, minimum and average area percent for components extracted with methylene chloride.

Component Name/ Retention Index	Average Area %	Minimum Area%	Maximum Area%
α-thujene	0.51	0.30	0.78
α-pinene	0.40	0.00	1.21
myrcene	2.40	1.60	3.82
octanal	0.09	0.01	0.65
α-phellandrene	0.36	0.00	1.13
RI-1008	0.19	0.00	0.88
β-E-ocimene	0.45	0.17	0.85
γ-terpinene	0.08	0.00	0.18
cis-linalooloxide	0.18	0.04	2.35
trans-linalooloxide	2.00	0.28	4.49
linalool	1.11	0.41	2.58
RI-1100	0.13	0.00	0.35
allo-ocimene	0.40	0.00	1.76
RI-1153	0.06	0.00	0.34
β-pinene oxide	0.19	0.00	0.64
nonanol	0.10	0.00	0.40
terpin-4-ol	0.48	0.04	1.07
RI-1192	0.18	0.00	0.93
α-terpineol	0.61	0.02	3.14
decanal (n)	0.61	0.23	1.83
trans-carveol	0.31	0.00	1.57
carvone	0.15	0.00	0.35
RI-1270	0.17	0.00	0.76
RI-1282	0.29	0.00	1.13
RI-1299	0.08	0.00	0.36
undecanal	0.13	0.00	0.57
RI-1323	0.24	0.00	0.65
α-terpinyl acetate	0.16	0.00	0.39

Table 2. -- continued

Component Name/ Retention Index	Average Area %	Minimum Area%	Maximum Area%
RI-1367	0.10	0.00	0.28
α-copaene	0.35	0.15	0.54
RI-1426	0.19	0.00	0.77
caryophyllene	7.60	0.88	15.11
α-humulene	0.66	0.09	1.29
germacrene	0.16	0.00	0.49
β-bisabolene	0.22	0.00	1.59
selinene	0.44	0.00	1.09
RI-1535	0.35	0.00	1.04
RI-1553	0.05	0.00	0.20
RI-1564	0.09	0.00	0.26
RI-1613	0.05	0.00	0.15
RI-1648	0.14	0.00	1.48
selin-11-en-4-a-ol	0.08	0.00	0.32
methyl jasmonate	0.08	0.00	0.29
RI-1677	0.12	0.00	0.29
cadinol	0.34	0.00	1.11
RI-1699	0.31	0.00	1.38
8,9-didehydronnootkatone	0.30	0.00	0.83
aristolene	0.17	0.00	0.74
RI-1796	0.12	0.00	0.63
RI-1810	0.13	0.00	0.44
nootkatone	6.70	1.68	17.82

Sensory Analysis

For comparison purposes all juices were ranked on the hasis of average hedonic preference score and divided into three approximately equal categories. There were ten juices in the "low" category. Average hedonic scores were 4.75 or helow. There were nine juices in the "medium" category. They had preference scores hetween 4.75-5.75. The 10 juices in the highly preferred category were rated ahove 5.75.

Sensory judgements of the panel were limited to a simple hedonic score hased on degree of like or dislike (preference). It should he kept in mind that the score for each juice represents preference rather than defined flavor. This could cause some scatter in sensory scores as some panelists might respond to different flavor aspects than others, nevertheless the majority of the panel typically responded in a similar fashion. Some of the scatter is reduced as the highest and lowest scores are typically discarded hefore the remaining scores are averaged. This sensory approach was chosen as it more closely reflects marketplace consumer attitudes.

Statistical Analysis

Univariate analysis

Tahle 3 shows the univariate correlations hetween preference scores of the panelists and individual peak areas. Correlation coefficients for individual components were low, ranging from 0.42 to -0.62. Myrcene, decanal, linalool, linalool oxides and several unidentified peaks were found to correlate negatively with sensory preference.

Table 3. Univariate correlations of selected volatile and non-volatile data with preference category.

Variable	Correlation (r)
allo-Ocimene	0.42
β-Caryophyllene	0.27
α-Humulene	0.22
RI-954*	0.21
Brix/Acid	0.18
Limonin	-0.02
Nootkatone	-0.14
trans-Linalool oxide	-0 39
γ-Terpinene	-0.42
Naringin	-0.47
Linalool	-0 49
Acid	-0.51
Decanal	-0 53
RI-1796*	-0.57
RI-935*	-0.61
Myrcene	-0.61
RI-1047*	-0.62
Brix	-0.67
*RI-Kovat's retention indices	

Correlation coefficient for *trans*-linalool oxide was -0.39. Pino et al. (1986 a) also reported that the linalool oxides correlated negatively towards grapefruit flavor preference.

β-caryophyllene, α-humulene and several unidentified peaks correlated positively witb sensory preference. In contrast, Pino and co-workers reported tbat methyl butyrate, ethyl butyrate, decanal, and nootkatone correlated positively with sensory preference. Since the methylene chloride extraction and concentration procedure was used in our study, metbyl and ethyl butyrate were not adequately extracted. Tberefore, a direct comparison with Pino's results could not be made. Nootkatone, an important component for grapefruit flavor (Stevens et al., 1970) did not correlate with sensory preference (r=-0.14). This strengthens the argument that a multivariate approacb should be taken for flavor analysis, since flavor is a perception of a combination of many components. Multivariate analysis takes several components into consideration at one time while establishing the relationship of one component to the overall flavor.

In terms of non-volatiles, the bitter naringin and sour total acid correlated negatively with preference (r=-0.47 and -0.51). However, there was no significant correlation of bitter limonin (r=- 0.02) with preference. Similar findings were reported by Pino and Cabrera (1988). However, earlier studies (Rouseff et al., 1980 ; Barros et al., 1983) found significant negative relationship between limonin and preference.

Multivariate analysis

Principal component analysis (PCA). PCA can be used to determine the inherent structure of the data and identifies the most differentiating variables within the data set as a whole. Variables or measurements which help to separate the data points are given more weight or emphasis. This weighting system is usually expressed as a loading factor. The larger the loading factor, the more differentiating the measurement. The results of the combined data set for the first three principal components are shown in Figure 6 a and 6 b. The first three eigenvectors accounted for 66 % of the total variance of the data. As seen in these figures, the highly preferred juice samples were tightly clustered but not completely separated from the low and medium preference juices. In general, the most preferred juices had the lowest PC 1 eigenvector values. The second principal component axis was not especially effective in separating the three categories of juices. In principal component 3, the highest preferred juices had eigenvector values close to zero. The least preferred juices had negative eigenvector values and the medium preference juices had positive values. The loadings in PC3 are not easy to interpret. As indicated earlier, the highly preferred juices had eigenvalues very close to zero. Thus the *balance* between negative and positively loaded measurements will be associated with preference. For example, α-humulene and acid have equal but opposite loadings and could contribute to an eigenvalue of approximately zero.

Component analysis. PCAs are typically calculated in the *correlation* mode. However, it is also possible to employ PCA in the *covariance* mode. In this mode, those non-redundant measurements which can best account for the maximum variance in the

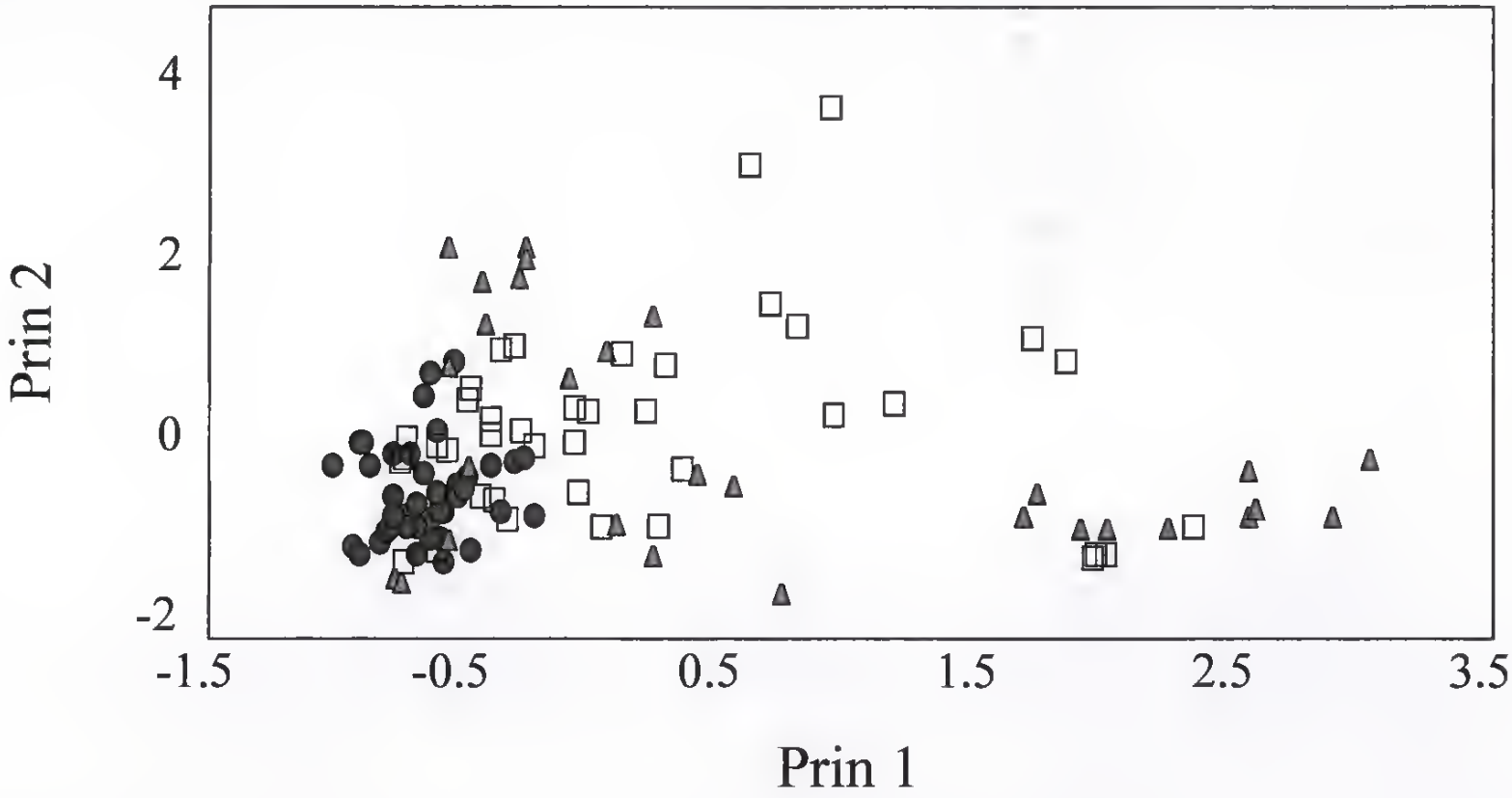


Figure 6a. Eigenvector values of PC1 vs PC2 from principal component analysis of all 57 volatile and taste components: (●) high preference category, (□) medium preference category, (▲) low preference category.

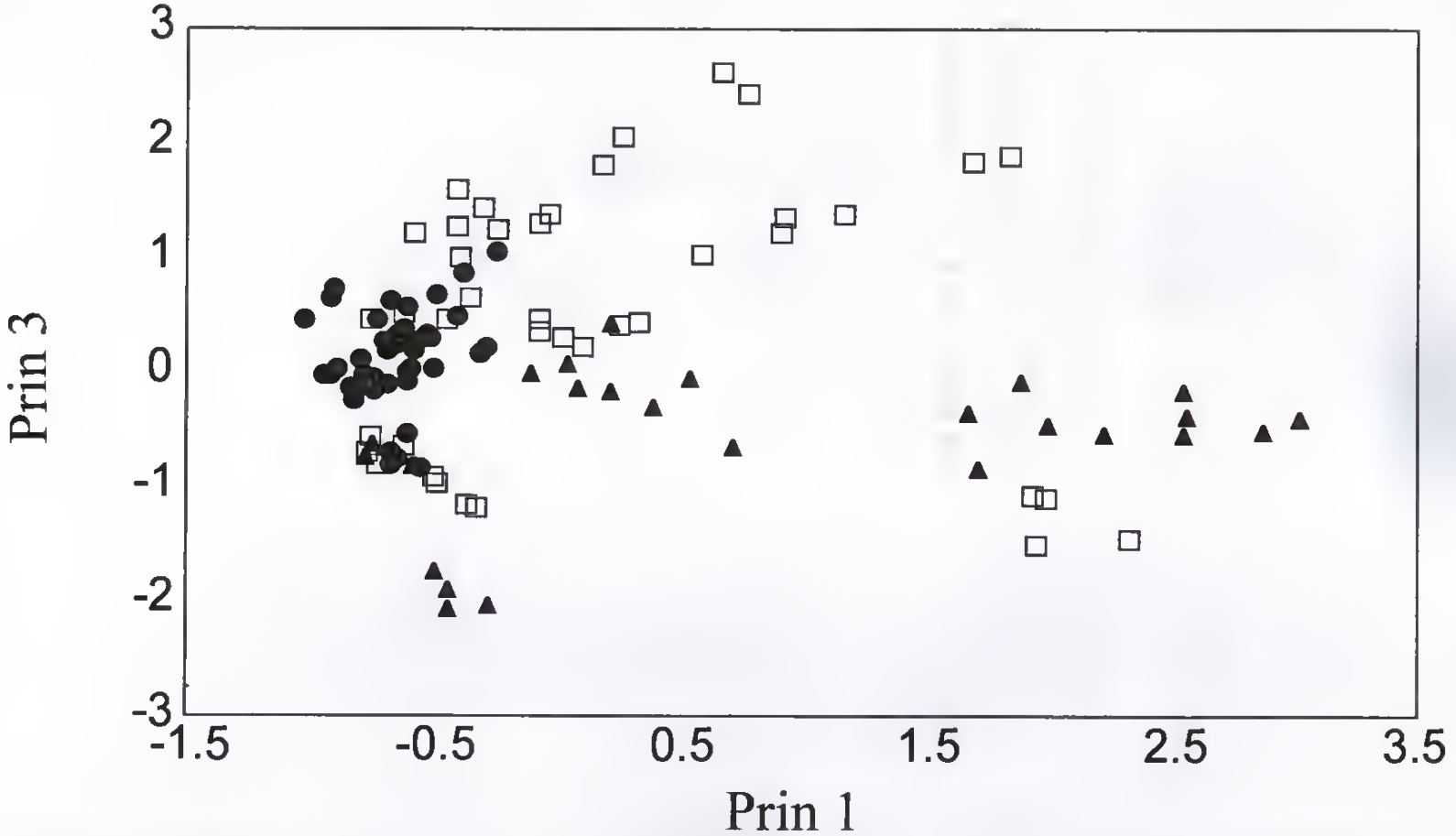


Figure 6b. Eigenvector values of PC2 vs PC3 from principal component analysis of all 57 volatile and taste components: (●) high preference category, (□) medium preference category, (▲) low preference category.

data, are given maximum loading. In the covariance mode, PCA 1 the loading is almost exclusively in favor of nootkatone (0.95). This indicates that nootkatone is one variable that can account for much of the variance in the data regardless of preference category. PCA 2 most heavily loads β-caryophyllene (0.94) whereas the loading in PCA 3 is weighted between myrcene and linalool (0.88 and 0.30 respectively). Essentially 97 % of the variance can be explained with these three eigenvectors. These compounds may be highly effective in accounting for the variance in the total data set, but they may or may not be effective in discriminating between samples in the three preference categories.

In order to determine if these four components might also discriminate with respect to preference category, the univariate correlation coefficients were compared from Table 3. It can be seen that nootkatone, which was effective in accounting for the total variance in all samples, was almost completely ineffective in differentiating between juices of various preference categories. On the other hand, myrcene which was also effective in accounting for total variance between all samples, was reasonably effective, ($r = -0.61$) in differentiating between juices of various preferences. Of the four measurements that accounted for most of the variance in the total data set, myrcene, β-caryophyllene and linalool were also effective in differentiating between juices of various preference. In Figures 7 a and b, various combinations of the peak areas for these three components are plotted against each other. It can be seen that essentially the same degree of separation between juices of various flavor preference using peak areas from these three compounds was achieved from the eigenvector value plots from all 57 components shown in Figures 6 a and b.

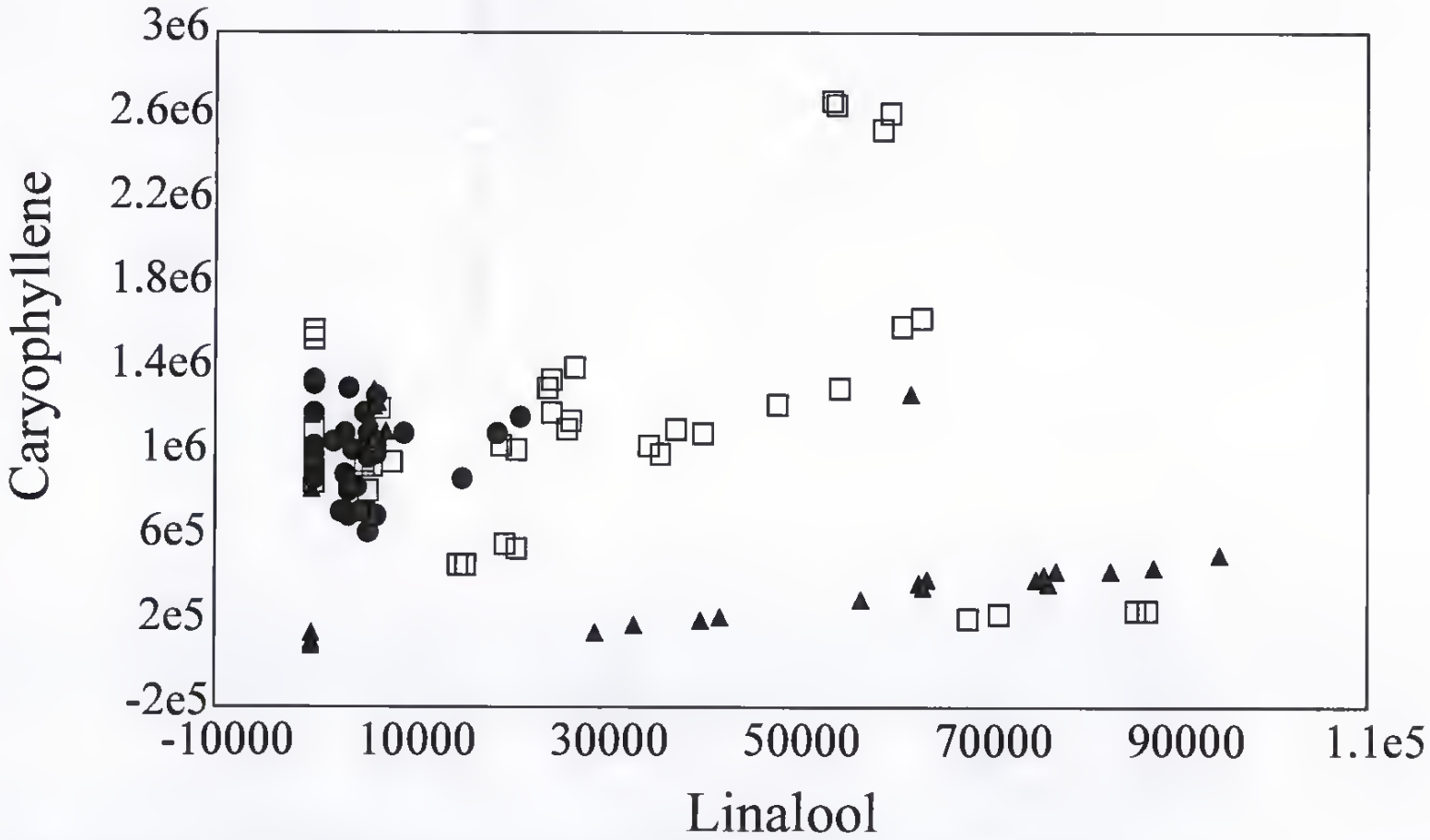


Figure 7a. Peak areas of linalool and caryophellene from 29 grapefruit juice extracts analyzed in triplicate: (●) high preference category, (□) medium preference category, (▲) low preference category.

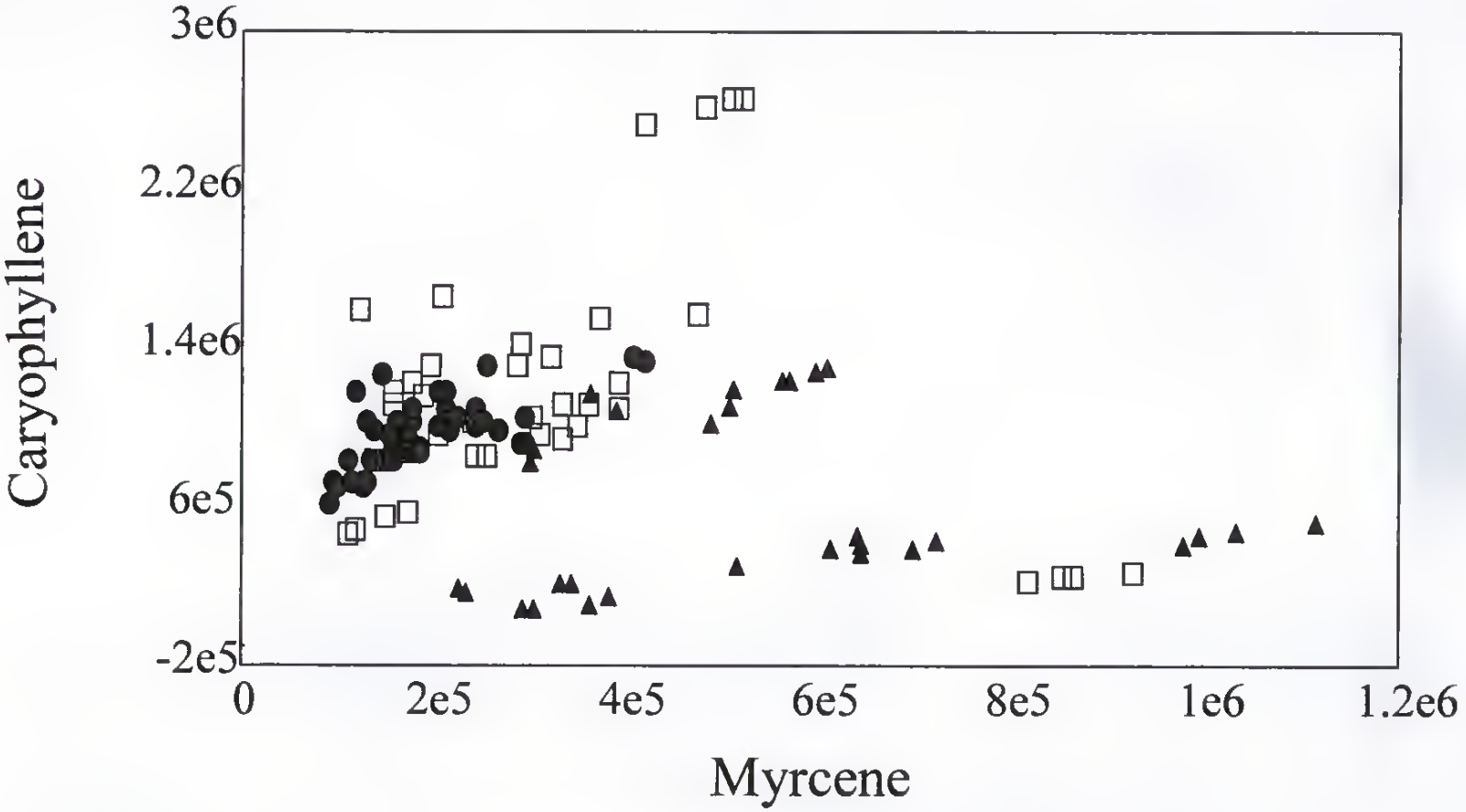


Figure 7b. Peak areas ofmyrcene and caryophellene from 29 grapefruit juice extracts analyzed in triplicate: (●) high preference category, (□) medium preference category, (▲) low preference category.

Nootkatone was not a particularly discriminating variable in this study. Our observed lack of nootkatone correlation agrees with the report of Shaw and Wilson (1981) and Pino et al. (1986 a, b). The indication that a high °Brix (sweetness) was strongly associated with the least preferred juices was unexpected. This suggests however, that highly sweet juices were not preferred. Finally, in identifying the components which correlate with highly preferred grapefruit juice, it is important to acknowledge that these components only correlate with preference, but may or may not be causative.

Discriminant analysis. In order to identify the variables which are most differentiating with respect to preference, discriminant analysis was used (Table 4 and 5). Discriminant analysis will load heavily those measurements which most effectively distinguish between juices of different preference category. Figure 8 a illustrates the results of discriminant analysis using just five components. All three preference category juices are clustered but several highly preferred samples have overlapped with the mid preference juices and four mid preference juices are found in the region of the low preference juices. However, increased category separation can be achieved if additional terms are used . Figure 8 b illustrates the separation which can be achieved with 13 components. One of these components was the peak *allo*-ocimene, the others are noted in the legend. This is the minimum number of components required to achieve 100% separation between juices of different flavor preference.

Table 4. Forward stepwise discriminant analysis (methylene chloride extractions).

Variable Name	Partial R**2	Wilk's lambda
Brix	0.46	0.54
RI-1677	0.29	0.38
α-Terpineol	0.23	0.29
β-Gurjunene	0.17	0.24
Ratio	0.15	0.21
Limonin	0.13	0.18
cis-Linalool Oxide	0.14	0.15
Naringin	0.24	0.12
Nonanal	0.23	0.09
Acid	0.14	0.08
allo-Ocimene	0.14	0.07
α-Copaene	0.15	0.06

Table 5. Discriminant analysis classification results (methylene chloride extracts).

Group	Compound	No. of Comp.	Percent Correct			
			Total	Low	Medium	High
B	Linalool +Myrcene	2	68	50	50	95
C	B+°Brix	3	78	83	53	98
D	C+β-Caryophyllene	4	78	90	58	89
E	D+Nootkatone	5	82	90	75	82
F	Linalool +Myrcene	2	68	50	50	95
G	F+°Brix	3	78	83	53	98
H	G+RI-1677	4	86	77	83	95
I	H+*allo*-Ocimene	5	90	97	80	95
Stepwise (backward)	16 components	16	100	100	100	100
Stepwise (forward)	19 components	19	100	100	100	100

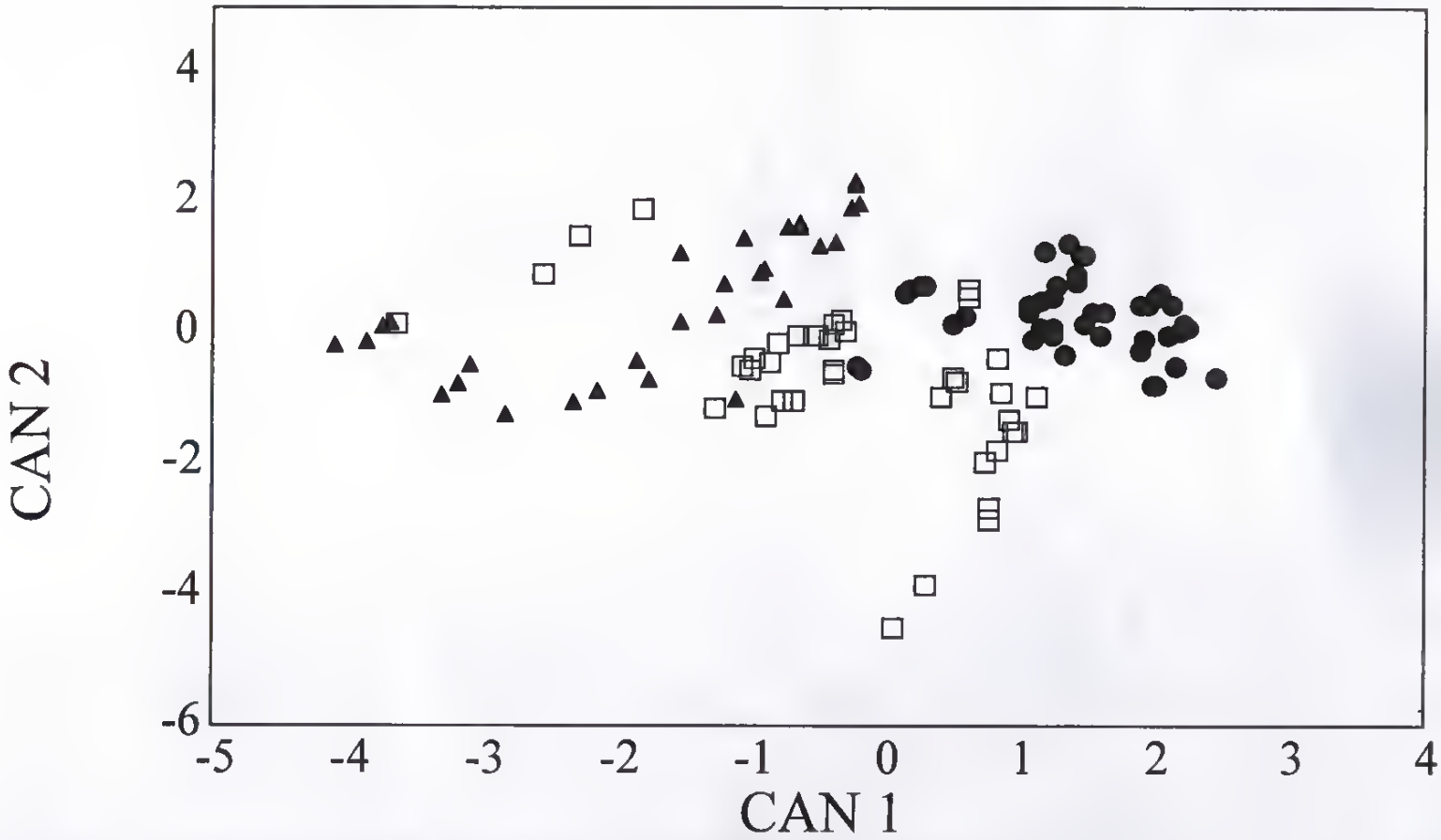


Figure 8a. Canonical discriminant analysis using myrcene, linalool, Brix, and peaks at RI-1677 and 1126: (●) high preference category, (□) medium preference category, (▲) low preference category.

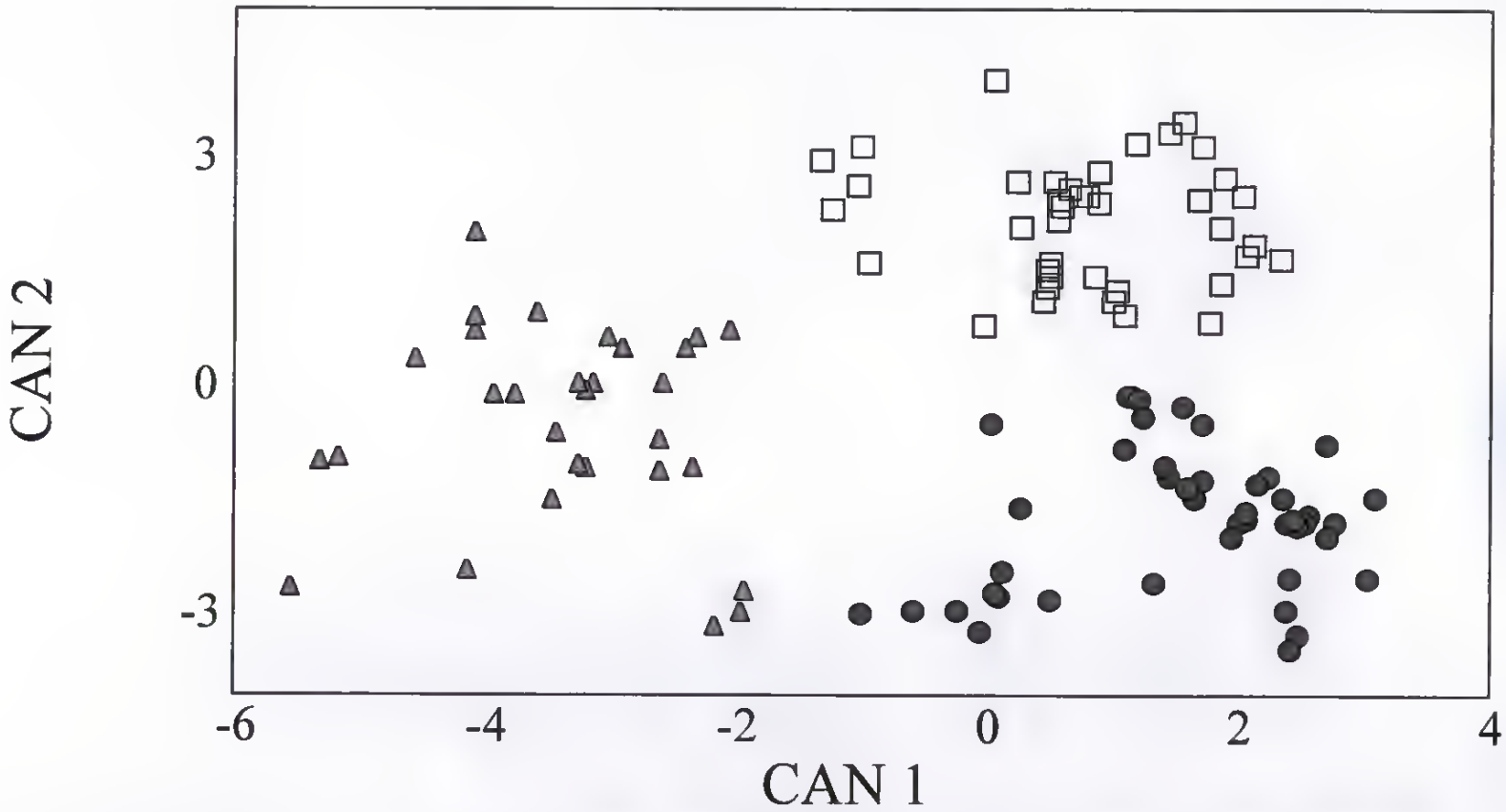


Figure 8b. Canonical discriminant analysis using 13 variables (ratio, RI-935, cis-linalooloxide, nonanal, allo-ocimene, a-terpineol, decanal, RI-1299, a-copaene, b-gurjunene, RI-1762, and RI-1796):
(●) high preference category, (□) medium preference category, (▲) low preference category.

Identification of the Peak at RI-1126

The peak with a Kovat's index value of 1126 was the single highest positively correlated component among the entire 57 components evaluated. GC-MS was employed to identify this peak. It was noted that the mass spectra at the front of the peak differed from that of the back half. Upon further examination, we found there was a major ion mass of 121 which was evident only during the first portion of the peak and a second major ion mass of 117 which could be seen only during the last half of the peak. This strongly suggested the single peak at the retention index 1126 consisted of two co-eluting compounds. When this peak was re-plotted as two single ion chromatograms, one generating using only the mass of 117 and the second using only the mass of 121, two distinct peaks were observed. By judiciously choosing the mass spectral scans spanning the elution time of the second compound for averaging with the background chosen as the mass spectral scans spanning the elution time of the first compound, it is possible to achieve a mass spectrum that is essentially free from ions due to the co-eluting compound. The same procedure can be repeated to produce library searchable spectra for both compounds. For the second peak the following spectrum was observed: m/z 121, 100 %; 105, 53.32 %; 136, 49.03 %; 91, 35.55 %; 79, 27.92 %; 93, 20.65 %; 77, 15.36 %; 19, 11.91 %; 22, 9.73 %; 103, 8.88 %. A library search (Adams, 1995) produced a match for the second peak that had a purity, fit, and rfit of 919, 944, and 954 respectively with *allo*-ocimene (2,6-dimethyl 2,4,6-octatriene). Not only is the mass spectrum a good match to the library spectrum, but the library spectrum has included with it a Kovat's

retention index (RI) for each compound. The library RI for *allo*-ocimene was 1129 which very close to the observed 1126. Therefore, designation is based on two independent means of identification.

The identification of the first eluting peak was more difficult. Its mass spectrum consisted of: m/z 43, 100.00 %; 117, 96.22 %; 71, 67.73 %; 89, 44.04 %; 55, 41.18 %; 69, 28.59 %; 41, 22.63 % 42, 21.47 %; 97, 21.00 %; 75, 18.76 %. The two best mass spectral matches were hexyl n-hexanoate and butyl n-hexanoate. However, these two compounds have RI values of 1383 and 1188 which were too high to be considered a match. The mass spectra for these esters along with the unknown peak all have a m/z 117 ion as a base peak which is from the common hexanoic acid part of the ester. The unknown spectrum contains a m/z peak of 43 which is indicative of a propyl fragment. The unknown also contains a m/z 159 ion which could be from a protonated propyl hexanoate ester. Also, the RI of 1126 would fit the pattern of decreasing RI's for decreasing size of the alcohol portion of the ester. For these reasons, we have suggested the first eluting compound might be propyl hexanoate (MW = 158).

This part of the study utilized the components which had highest correlations for predicting the juice quality. However, these correlated components may or may not be causative for the over all flavor quality of the juice. More-over, methylene chloride did not efficiently extract the top note volatiles. Since the top notes were proven to contribute to the aroma quality (Marin et al., 1992; Bazemore, 1995; Hinterholzer and Schieberle, 1998), further studies were done to investigate the optimum solvent and use of human responses with GC-olfactometry.

Grapefruit Juice Aroma Extraction Methods

Isolating and analyzing the volatile components of a food product is essential due to their significant contribution to overall flavor. Comparison of volatile component isolation procedures have been reviewed by several researchers (Weurman, 1969; Nunez et al., 1984; Moshanas and Shaw, 1982 &1992) . The purpose of this portion of the study was to establish the most representative extraction technique for grapefruit juice aroma components. The three methods evaluated here are: liquid-liquid extraction, dynamic head space purge and trap solvent elution, and static head space extraction using SPME. These extraction methods have been used earlier in citrus juices. Moshanas and Shaw (1982) and Nunez et al. (1984) have assessed liquid-liquid extraction in orange and grapefruit juice respectively. Dynamic head space thermal desorption has been used in orange juice by Moshonas and Shaw (1992) and in grapefruit juice by Cadwallader and Xu (1994).

Chromatographic Separation and Analysis

Capillary gas chromatography is the best technique to separate the volatile components in grapefruit juice. In this technique, components are eluted based on their boiling points and the peak areas are proportional to the components present in the sample. Figure 9 represents a typical chromatogram for grapefruit juice. It can be roughly divided into 4 regions:

1. top notes-- includes very volatile components such as ethanol, acetaldehyde, hexanal,

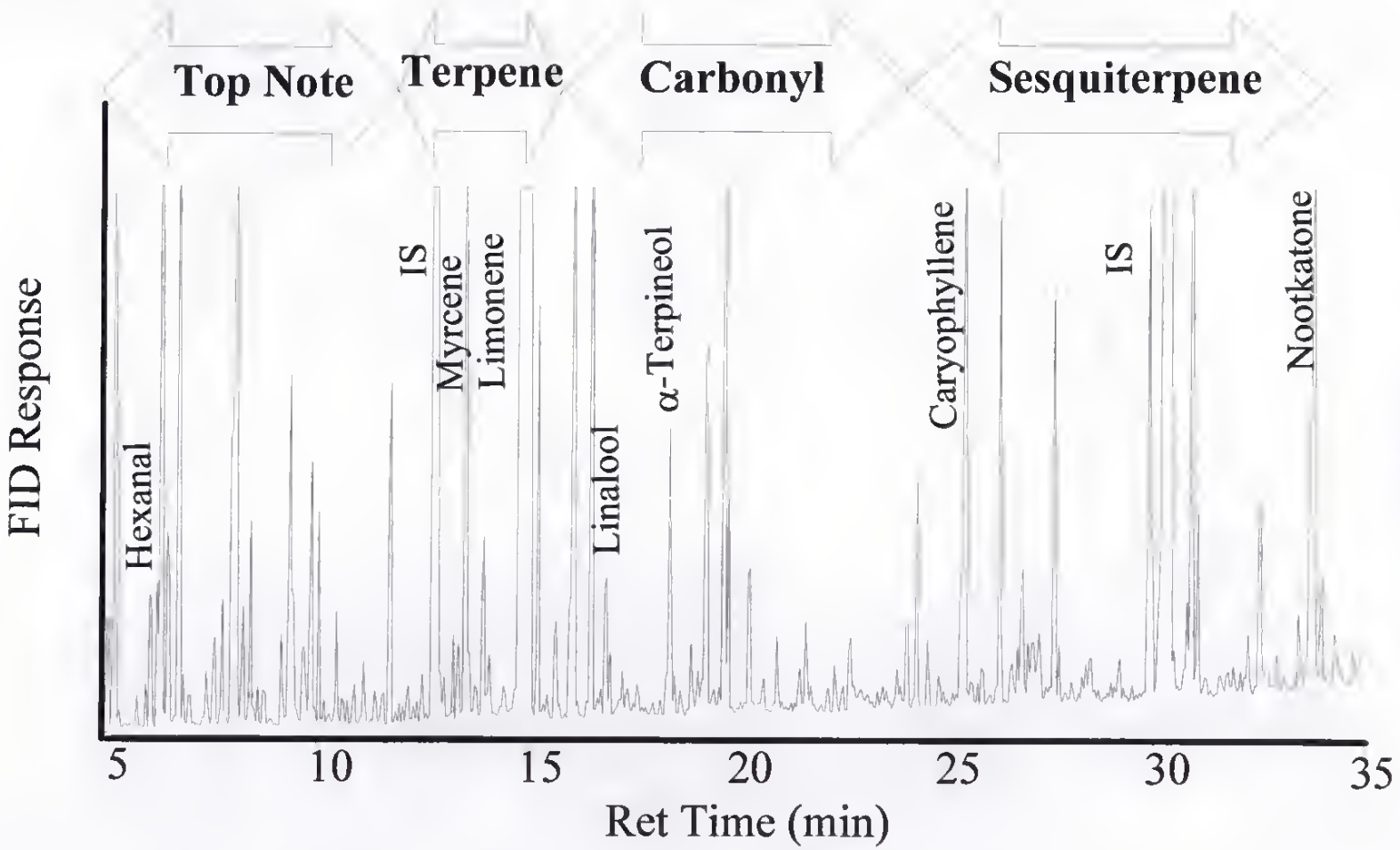


Figure 9. Chromatogram classification of pasteurized grapefruit juice (pentane-ether extracts).

2. terpene area-- includes components like limonene, myrcene, sabinene,
3. carbonyl region-- consists of octanal, nonanal, terpene alcohols and oxides,
4. sesquiterpene area-- includes components like caryophyllene and nootkatone.

Extraction Methods

There is no single extraction method which can extract all the aroma components in the exact proportion they exist in the sample. Each procedure will concentrate some components and to varying degrees discriminate against others. Since the aroma active components in grapefruit juice range from low boiling top notes to high boiling sesquiterpenes, one of the goals of this study was to optimize extraction procedures so as to obtain the most representative aroma profile for grapefruit juice. Individual components were quantified to facilitate comparison between extraction procedures. Figure 10 compares the representative chromatograms obtained by different extraction techniques. Table 6 summarizes analytical precision in terms of percent relative standard deviations (% RSD)of the extraction methods for major juice components.

Liquid-liquid extractions

Pentane/diethyl ether (1:1) liquid-liquid extraction isolated a wide range of components ranging from top notes to sesquiterpenes. In the earlier section, methylene chloride was used as the solvent to extract aroma components. The relative absence of low boiling early eluting components is shown in Figure 5. Table 7 compares the peak areas obtained from the top note region. Pentane-diethyl ether extractions yielded 73 %

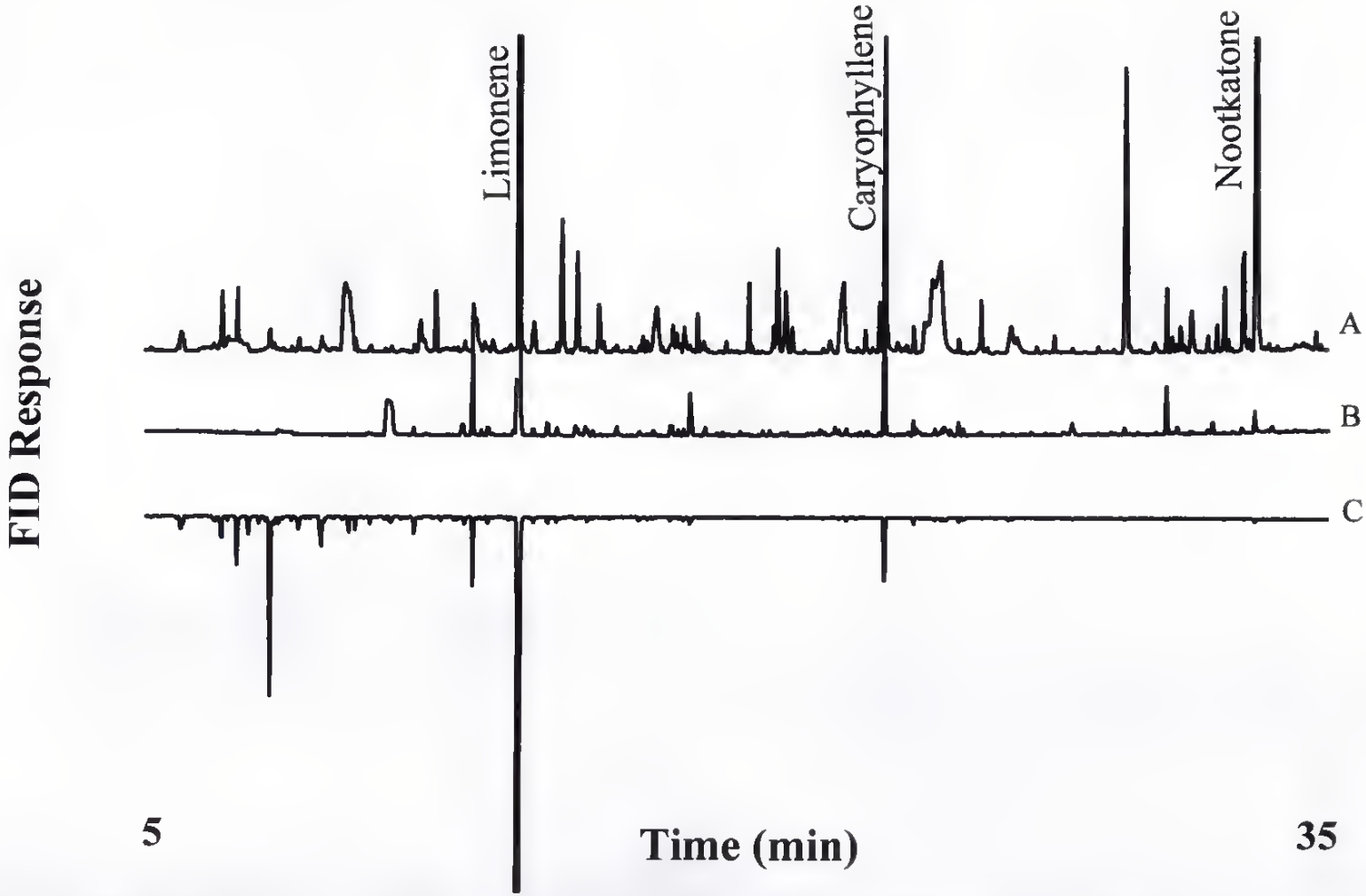


Figure 10. Aroma extraction methods in grapefruit juice: A)liquid liquid extraction (pentane ether 1:1), B) static headspace extraction (solid phase microextraction-SPME), C) dynamic headspace purge and trap solvent elution (Tenax/charcoal trap).

Table 6. Percent relative standard deviation for different aroma extraction methods in grapefruit juice.

Component	% RSD	
	Pentane-Ether	Dynamic HS
Hexanal	10	7
α-Pinene	13	8
Myrcene	3	3
α-phellandrene	9	18
cis-linalool oxide	8	5
trans-linalool oxide	4	16
allo-ocimene	10	ND
α-terpineol	16	ND
Terpin-4-ol	12	ND
Caryophyllene	10	3
α-Humulene	7	ND
Nootkatone	8	ND

Table 7. Topnote peak areas for different aroma extraction methods.

	Peak Areas		
	Liquid-liquid		
Kovat's Indices	MeCl	P&E	Dy-HS
RI-801		2,785	13,872
RI-805			10,925
RI-814	913	49,342	421,594
RI-821	6,149	3,929	
RI-834	2,318	16,318	34,206
RI-840		7,199	
RI-844		15,610	8,495
RI-854			94,108
RI-872	10,612		13,347
RI-877			44,674
RI-891			3,256
RI-897	6,145	8,103	12,443
RI-909	4,986	5,364	5,434
RI-915		8,167	20,843
RI-924		10,128	10,472
RI-936	76,251	10,660	43,638
RI-941	39,368	106,486	
RI-944	10,737		
RI-965	1,809	31,574	8,307
RI-971	6,800		12,518
RI-982	7,087	24,217	22,148
Total top note peak area	173,175	299,882	780,279

more top note peak area than methylene chloride. Total top note peak area obtained from dynamic head space analysis was 350 % more than the liquid-liquid methylene chloride extracts. Preferential selectivity of methylene chloride for non-polar components in citrus juices was also reported by Nunez et al. (1984) and Moshonas and Shaw (1982). Since aroma active components in the top note area, like ethylbutyrate, hexanal, were efficiently extracted by pentane-diethyl ether, it was utilized as the extraction solvent for this study. Nunez et al. (1984) also used pentane-diethyl ether solvent mixture for extracting grapefruit juice aroma components, but no quantitative data were presented in their study. However, extraction of a wide range of components with a wide range of polarity by a mixture of pentane-diethyl ether solvents for grapefruit juice has been reported by that author. Lower percent relative standard deviations were observed for most components in pentane-diethyl ether extractions (Table 6). To our knowledge, there are no previous reports which provide extraction reproducibility utilizing liquid-liquid extraction for the volatile components in grapefruit juice.

Dynamic head space extraction

Dynamic head space involves the continual movement of volatiles from the bulk of the sample into the gaseous phase where it is swept into a trap (Wampler, 1997). The sample volatiles are constantly swept by a flow of carrier gas and a state of equilibrium between sample matrix and head space is never reached. This increases the volume of head space gas beyond the limit of the head space in the sample vessel. Volatiles must be collected on a trap and can be used for subsequent analysis. In this study, a mixture of

charcoal and Tenax® sorbent materials were used as adsorbents. These absorbents are commonly used for the isolation of volatiles (Buttery and Ling, 1996; Wampler, 1997). Tenax® is capable of trapping a wide range of organic volatiles but is not well suited for low molecular weight hydrocarbons and smaller alcohols (C1-C4). Charcoal, on the other hand, has affinity to collect small organic compounds and has higher retentive capacity.

Moisture can be a problem when trapping aroma volatiles. The use of sodium sulfate or purging the absorbents with inert gases are common methods found in the literature. Since grapefruit juice is approximately 90% water, the absorbents were purged with dry nitrogen to remove any trapped moisture.

Dynamic head space purge and trap solvent elution was effective in extracting top note volatiles (Figure 10). This method extracted 160 % more top note peak area than the pentane-diethyl ether liquid-liquid extractions. However, higher vapor pressure components like oxygenated mono and sesquiterpenes were not effectively purged from the sample. This means components thought to be important to grapefruit flavor such as nootkatone (Stevens et al., 1970) could not be quantified using this technique. Cadwallader and Xu (1994) reported similar results for dynamic head space analysis of grapefruit juice. However, they used cryotrapping and thermal desorption and were able to detect early eluting components such as ethanol and acetaldehyde which are normally obscured by the solvent peak. Percent RSD reported for our procedure was comparable to those reported by Cadwallader and Xu (1994). Since this method did not effectively extract the high boiling aroma active components, we did not use this method for further analyses.

Static head space extraction using SPME

Solid phase micro extraction is a rapid procedure to sample volatile components in head space gases. It involves the adsorption of head space volatiles onto a coated fiber which is exposed to the head space for a specific time. In the static head space method, volatiles in the sample matrix are allowed to come to an equilibrium with the head space before being sampled. The SPME technique is relatively new technique and has been used for analyzing orange essence volatiles (Bazemore, 1995), orange juice volatiles (Steffen and Pawliszyn, 1996), head space of milk powder (Stevenson and Chen, 1996), and cheese volatiles (Chin et al., 1996).

The SPME method effectively extracted terpenes such as limonene and myrcene (as they were the largest peaks in the resultant chromatogram) but was relatively ineffective in extracting the top note volatiles. The SPME fibers adsorb components on a competitive basis. Since terpenes (especially limonene) are in higher concentrations in grapefruit juice and also due to their non-polar nature, distribution coefficients and affinity of fiber to non-polar components, they tend to dominate the head space components trapped by the fiber coating.

Steffen and Pawliszyn (1996) reported good reproducibility for the components in orange juice. However, the authors centrifuged the samples prior to analysis, which eliminated the juice pulp and suspended solids. Lower levels of precision values were obtained when sampling was done on whole grapefruit juice (private communications - Bazemore, 1998). Since SPME emphasizes terpenes, which are in high concentrations but contribute little to aroma, this technique was not used for further analysis in this research.

GC-Olfactometry Studies

GC-olfactometry (GC-O) is an important analytical tool since it characterizes the odors of individual compounds and identifies which GC peaks have aroma activity (Mistry et al., 1997). A human nose is used to detect and evaluate the effluents from the column instead of an analytical detector. It is a powerful and sensitive tool since the odor detection limit of a human nose is 10^{-19} moles (Reineccius, 1994), which is considerably more sensitive than most instrumental detectors.

Grapefruit juice is a complex matrix and not all volatile components have aroma activity. Even among those components which have aroma activity, some will have more impact than others. Therefore, GC-O has been utilized to identify and characterize the odor active components in grapefruit juice extracts. Aroma active components in grapefruit juice change with the fruit maturity and also from thermal processing. In this study aroma extracts from unpasteurized and pasteurized juices from early, mid and late season fruits were evaluated for individual aroma active components.

Instrumental Detectors vs. Human Response

GC-O detects only those components which have aroma activity. Some of these aroma active components are very potent and are present in such small amounts that they cannot be detected by typical GC detectors. Figure 11 compares the consensus aromagram (aroma intensities of 3 panelists were averaged) produced by GC-O with

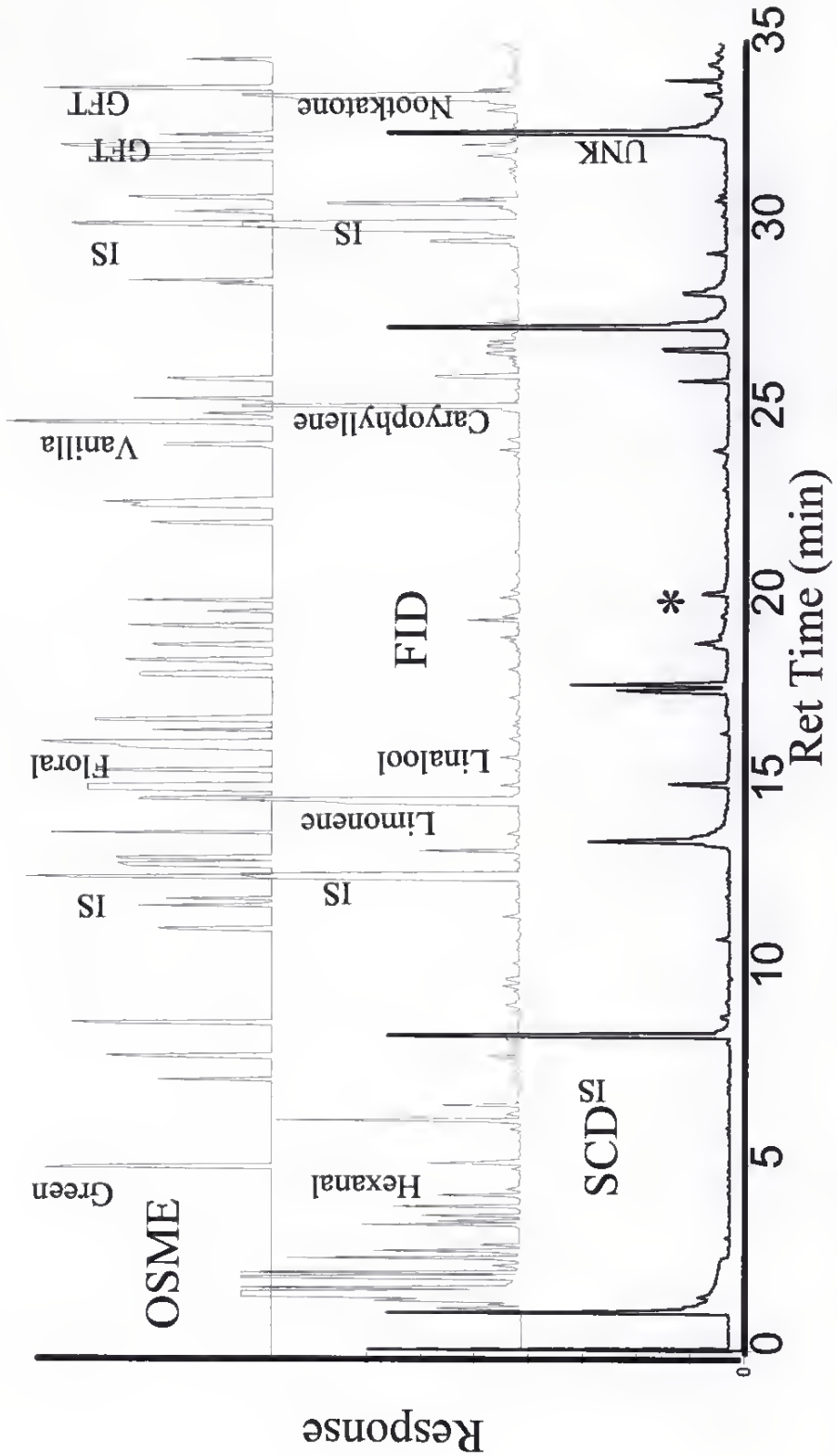


Figure 11. Comparison of aromagram from OSME and chromatograms from FID and SCD
*p-menenthene-8-thiol

chromatograms produced by FID and SCD detectors. The instrumental detectors responded to some components which the human nose did not recognize. Conversely, the human nose detected some compounds which gave no instrumental response. Large peaks in FID like limonene and caryophyllene seem to have little to no aroma activity. Panelists described limonene as citrusy, mediciny and minty with a moderate intensity, while they could not detect any aroma activity for caryophyllene. Earlier work by Marin et al. (1992) also reported a limited aroma activity of limonene in orange juice.

Among the small FID peaks, vanillin is notable. It was found to have intense vanilla or white chocolate aroma (average aroma intensity = 13). Vanillin has been reported for the first time in grapefruit juice by our group. A strong intense aroma peak was obtained at a 25 min retention time that has the characteristic aroma of vanillin (see Figure 11). The same grapefruit juice extract was analyzed using GC-MS for further confirmation of the presence of vanillin. By comparing the mass spectrum of the sample with the mass spectrum of the standard, it can be concluded that the peak with aroma attribute vanilla was, in fact, vanillin. The total ion chromatogram and the mass spectra of vanillin sample and the standard, are shown in Appendix A and B. Prior to this, vanillin was identified in orange juice by Marin et al. (1992). Peleg et al. (1992) proposed the path ways for formation of vanillin from ferulic acid in orange juice (Figure 12). According to the authors (Peleg et al., 1992), vanillin can form from ferulic acid through decarboxylation and oxidation or directly from free ferulic acid through retro aldol reactions. Similar reaction pathways may also occur in grapefruit. Intense aroma activity

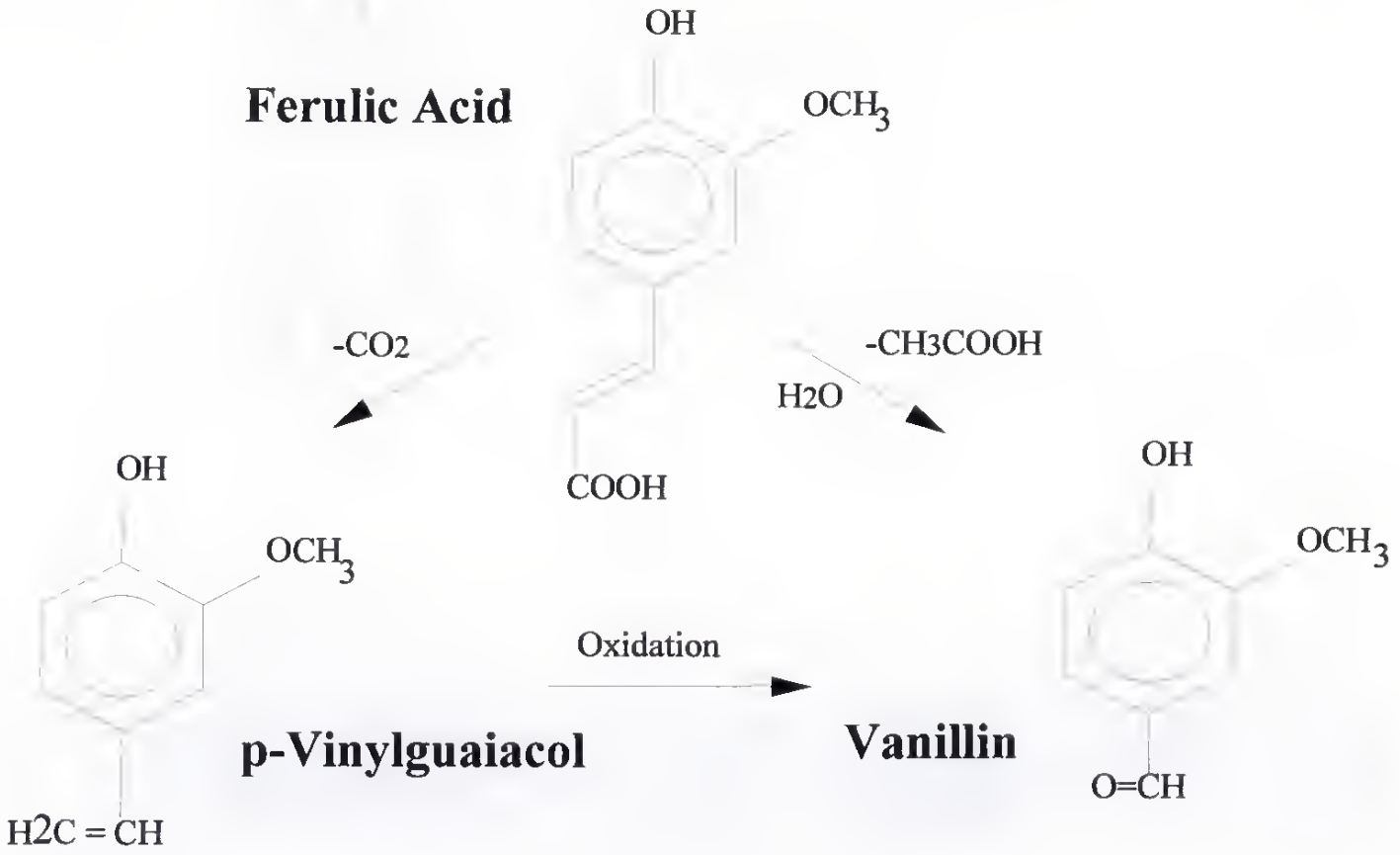


Figure 12. Formation of vanillin from ferulic acid.

of vanillin was also reported in oak aged wines (Aiken and Noble, 1984), Japanese green tea (Acree and King, 1996) and in coffee (Akieda and Kato, 1987).

Maturity and Processing Changes

In this part of the study, effects of maturity (early, mid and late) and processing (unpasteurized and pasteurized) are evaluated using GC-olfactometry. Fruit maturity as well as thermal processing affect the aroma quality of grapefruit juice. This is reflected in the differences in number and kinds of aroma active peaks detected in juices from different maturities. Figure 13a and b compares aroma attributes in early, mid and late season unpasteurized and pasteurized juices. A total of 37 - 49 aroma active peaks were found in early, mid and late season grapefruit juices. Appendix C lists the attributes perceived in juices of different maturities. Forty-one aroma components could be differentiated in early season unpasteurized juices while 37 peaks were detected in pasteurized juices. As a result of thermal treatment 11 aroma compounds were lost while 7 new components were formed in early season juice. However, many compounds were unchanged. Table 8 shows the aroma attribute compounds formed or lost during thermal processing of early season juices.

Mid season juices had 43 aroma active peaks in unpasteurized juice and 49 in processed juice. Similarly, 43 aroma active peaks were detected in both unpasteurized and pasteurized late season grapefruit juices. Eight components were lost in thermally treated late season juices, while 8 new attributes were detected. The aroma active peaks lost due

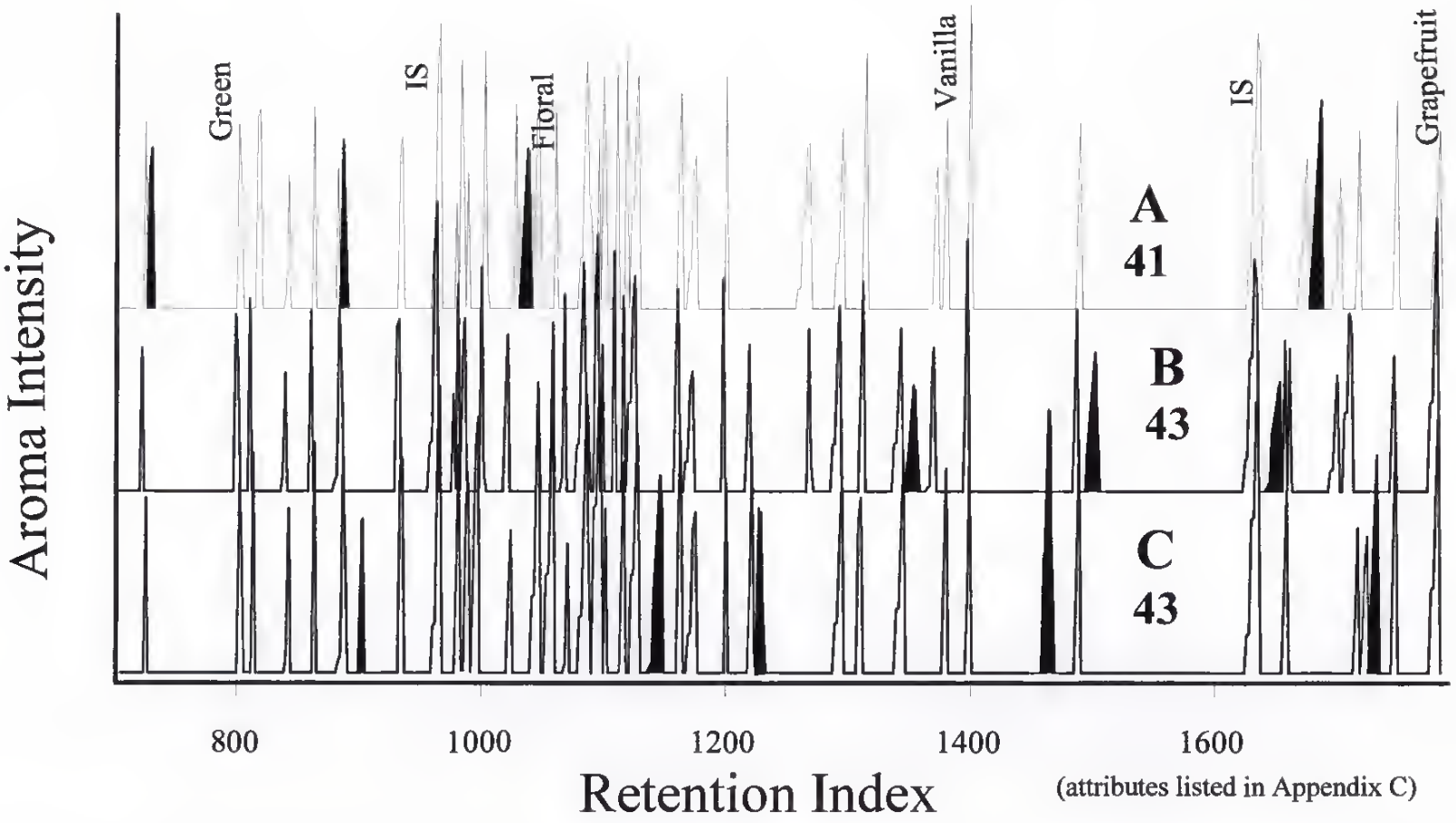


Figure 13a. Number of aroma active components at different maturities in unpasteurized grapefruit juice: A) early season, B) mid season and C) late season.

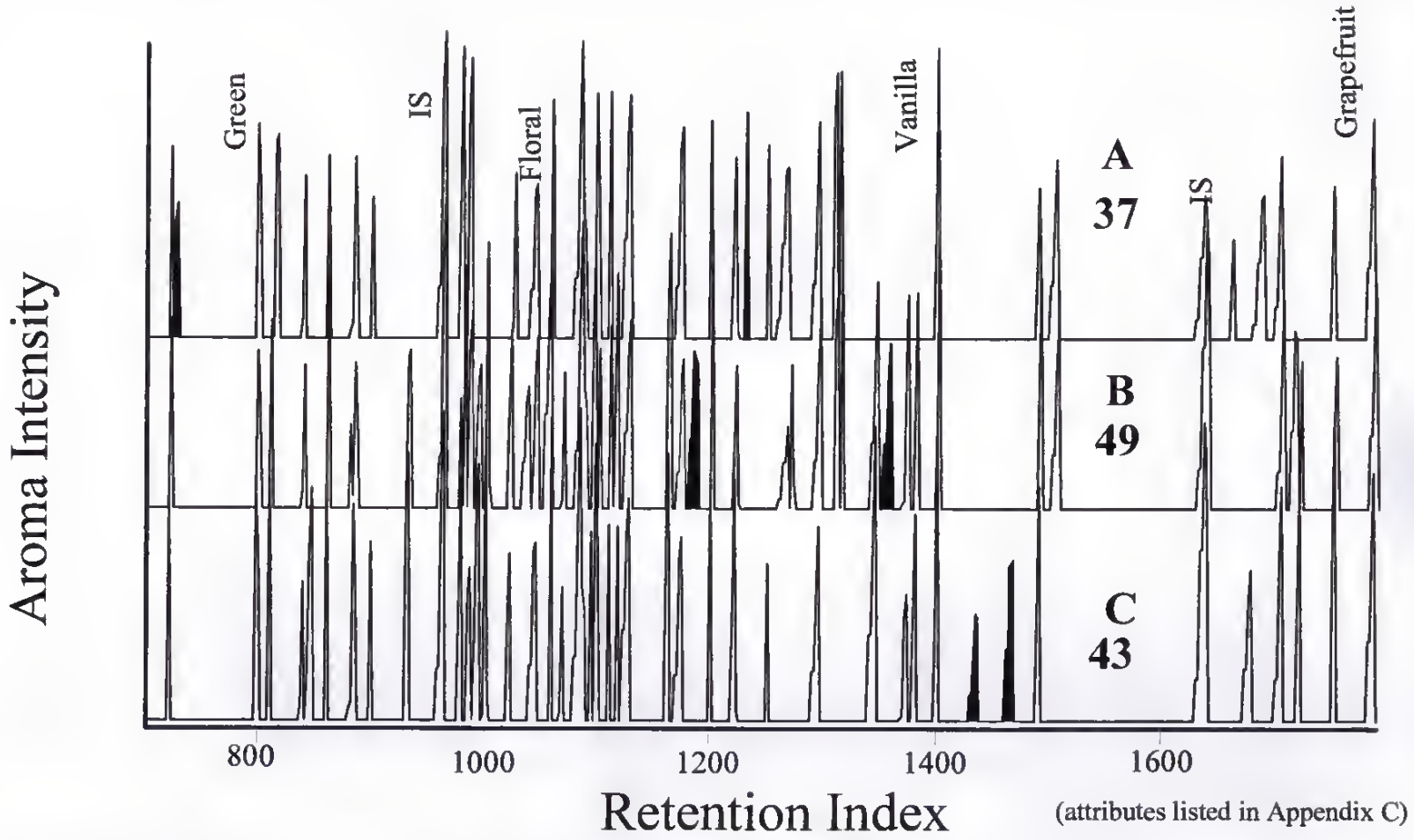


Figure 13b. Number of aroma active components at different maturities in pasteurized grapefruit juice: A) early season, B) mid season and C) late season.

Table 8. Formation and loss of aroma attributes due to pasteurization in early season red grapefruit juices.

Components/	Intensities		
Retention Indices	Pasteurized	Unpasteurized	Description
RI-896		7.0	Citrusy, Mediciny
RI-936	5.7		Floral, Smokey
α-Pinene		8 2	Greenish
α-phellandrene		12.3	Citrus
RI-1044		7.6	Rotten Fruit
RI-1095		10.3	Terpeney, Cucumber
RI-1116		12.7	Mediciney
RI-1166		10.4	Musty
RI-1217	7.3		Terpeney
RI-1223	9.3		Musty
RI-1227	7.8		Stinky fruit
RI-1318	10.8		Smokey, Rancid
RI-1374		6 8	Mediciney, Minty
RI-1381		9.2	Sweet
RI-1510	7 2		Spicey, perfumey
RI-1662	4 0		Peppery
RI-1684		7.2	Pungent
RI-1723		8 6	Rotten Grapefruit

to pasteurization had generally favorable sensory attributes like green, fruity while the components formed as a result of heating had roasted, fruity, and spicey attributes.

Concentration of the components also changes due to maturity and thermal processing Total alcohols, aldehydes and hydrocarbons were higher in early season unpasteurized juice (Figure 14a). Among the alcohols, α-terpineol, terpin 4-ol, *trans* linalool oxide, and among the hydrocarbons, myrcene and γ-terpinene were found to correlate negatively with sensory preference of grapefruit juice (Jella et al., 1998). These negatively correlated compounds were present in higher concentrations in early season than in late season juices. Results of this are summarized in Table 9. The levels of these components in grapefruit juice are in concurrence with those reported by Maarse and Visscher (1989).

As a result of pasteurization, increased concentrations of alcohols, aldehydes and hydrocarbons were observed (Figure 14b). Higher levels of alcohols are probably due to acid catalyzed reactions of terpenes like limonene, β-pinene, myrcene and so on. These components react in dilute aqueous acid and high temperatures to give several reaction products, some of which are alcohols like α-terpineol, terpin-4-ol and linalool oxides (Clark and Chamblee, 1992 and Shaw, 1991). Limonene is the major terpene in citrus juices and readily forms several reaction products under the conditions present in citrus juices.

α-terpineol in pure form and at low levels has a lilac aroma (Arctander 1994). However, at higher concentrations it tends to have musty odor (Marcotte et al., 1998). The level of this component in early, mid and late season pasteurized juices are 0.81, 1.97

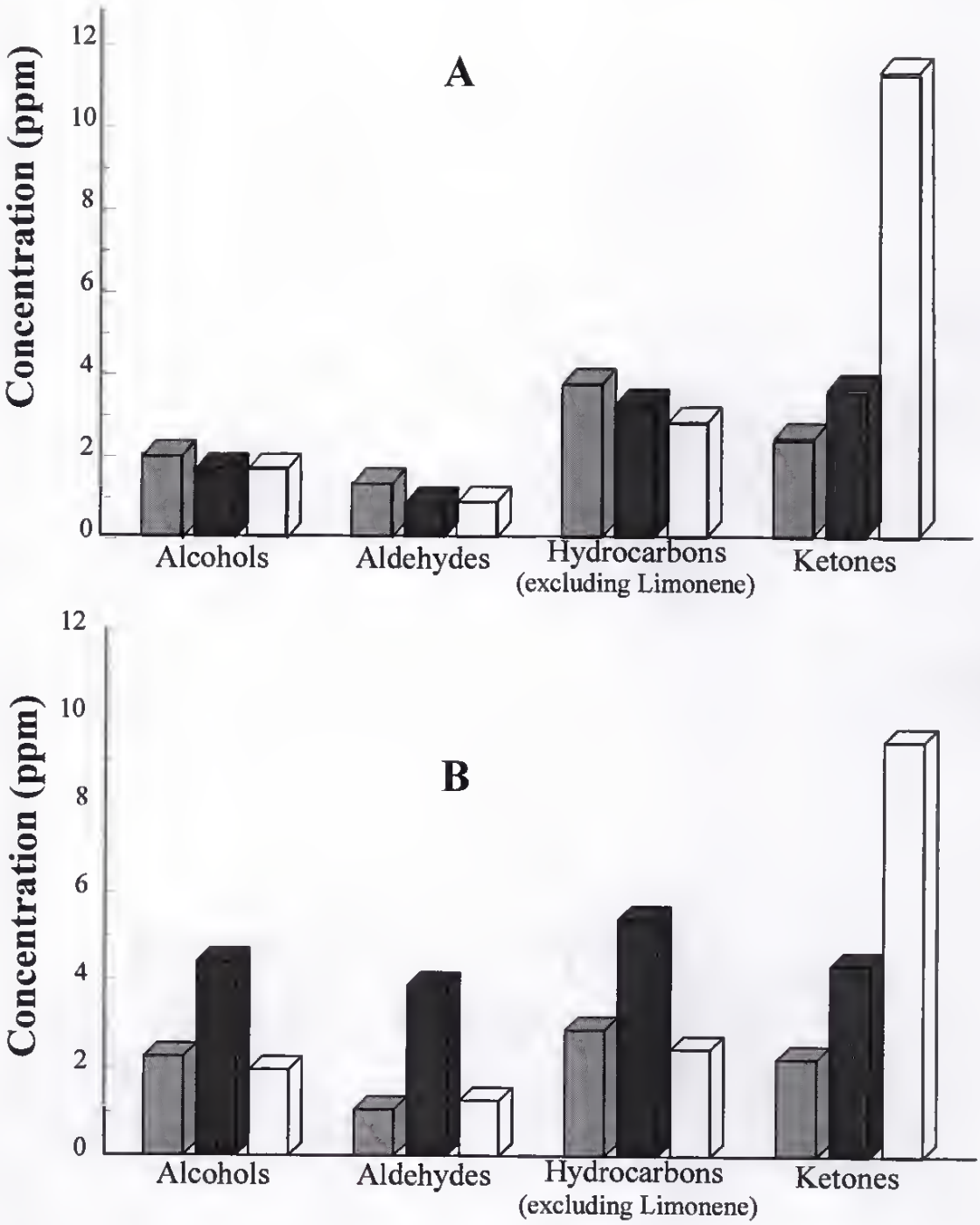


Figure 14. Concentrations of components in grapefruit juice.
A) unpasteurized juices; B) pasteurized juices: ( ) early season,
( ) mid season and ( ) late season

Table 9. Concentration levels (ppm) of components in early, mid and late season red grapefruit juices.

Component	Early Season		Mid Season		Late Season	
	Unpasteurized	Pasteurized	Unpasteurized	Pasteurized	Unpasteurized	Pasteurized
α-terpineol	0.343	0 809	0.228	1.967	0.242	0.614
Terpin-4-ol	0.174	0.171	0.126	0.194	0.168	0..215
trans-linalool oxide	0.426	0.450	0.420	1.461	0.295	0.311
Myrcene	1.790	1 371	1.788	2.942	1.166	1.049
γ-terpinene	0.263	0 260	0.189	0.284	0.260	0.218

and 0.61 ppm respectively. Panelists in this study described it as having "stale church" or "wet dog" smell. Limonene is reported to undergo acid catalyzed hydration to form α-terpineol (Clark and Chamblee, 1992) (Figure 15). Mid season unpasteurized juice had higher concentration of limonene (37 ppm) than early and late season unpasteurized juices (33 and 25 ppm respectively). Therefore, higher concentrations of α-terpineol can be expected in mid season juices.

Standard Descriptors Vs. Panelist's Descriptors

Linalool is described as having a strong floral aroma (Arctander 1994), and is an important contributor to the flavor and aroma of numerous products including lemon oil, certain teas (Clark and Chamblee, 1992) and orange juice (Marin et al., 1992). Other components having significant aroma contribution to orange juice are ethylbutyrate, hexenal, vanillin, octanal and nonanal (Marin et al., 1992; Bazemore, 1995; da Silva et al., 1994). Table 10 compares the aroma descriptors given by panelists for some of the components present in grapefruit juice. Because there is no standard lexicon, free choice descriptors were encouraged. Hence it was not surprising to see that for a single component the descriptors given by the panelists differed. Also, multiple synonymous terms were used by the panelists for one component. For example hexanal was described by the panelists either as green, grassy or herbacious. However, by comparing the elution times and Kovats indices for aroma active peaks, it can be concluded that the panelists were describing the same peak using a different descriptor. Table 11 compares the attributes described by the panelists with that of the standard descriptors given by

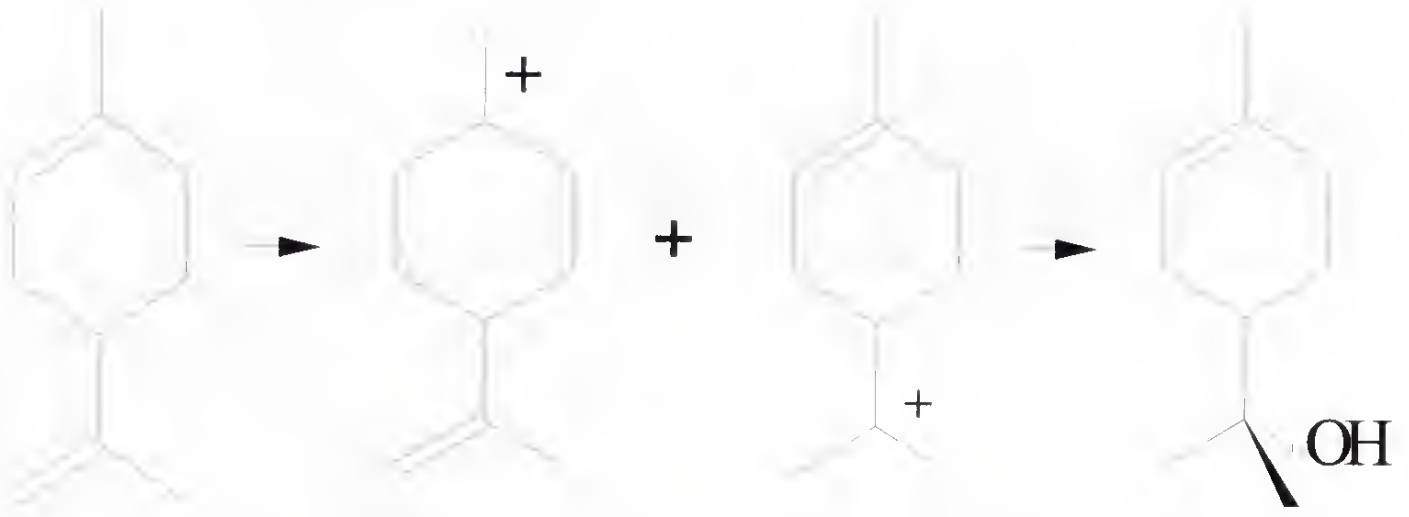


Figure 15. Acid catalyzed hydration of limonene

Table 10. Aroma descriptors used by panelists from GC-O experiments of citrus standards.

Component	Panelist 1	Panelist 2	Panelist 3
Hexenal	Green	Green	Strong green
Ethyl Butyrate	Fruity	Fruity, Floral	Fruity, Citrus
t-2-hexenal	Green, dead bug	Skunky	Smoked burnt roasted
α-Pinene	Medicine, Piney	Piney, Green	Greenish, Vitamin C
Myrcene	Unripe Mango	Melon	Citrus
Linalool	Floral	Linalool	Stinky floral
Terpin-4-ol	New cotton clothes	Stale Church	Vinyl
α-terpineol	Cilantro, Musty	Moldy, Musty	Green cilantro
p-menthene 8-thiol	Rotten gft, Stinky terpeney	Stinky Rotten GFT	Sweet grapefruit
Nootkatone	GFt stink, Rotten Gft	Moldy GFT	Stinky grapefruit
Benzaldehyde (IS)	Cherry, Almond	Cherry, Almond	Cherry, Almond
Methyl Jasmonate (IS)	Floral, Jasmine	Floral, Jasmine	Floral, Jasmine

Table 11. Comparison of standard (Arctander lexicon) with panelist descriptors.

Component	Standard Descriptor	Panelist's Descriptor
Hexenal, Ethyl Butyrate	Green/Warm sweet fruity	Green/Fruity
t-2-hexenal	Green vegetable like	Green, dead bug, skunky
α-Pinene	Warm resinous and herbacious	Medicine
Benzaldehyde	Bitter almond, sweet cherry	Cherry, almond
Sabinene	Warm peppery, herbacious	Unripe mango, piney
Myrcene	Balsamic resinous and citrusy	Unripe mango, citrus
α-phellandrene	Citrusy, peppery, woody	Citrus
para-cymene	Citrusy, kerosene like	Minty, citrusy
trans-β-ocimene	Warm herbacious, sweet	Citrusy, musty
γ-terpinene	Herbacious, citrusy	Roasted cotton candy
cis-linalool oxide	Sweet floral earthy	Terpeney, cotton candy
trans-linalool oxide	Sweet floral earthy	Terpeney, cotton candy
Nonanal	Fatty, floral	Terpeney, cucumber, cotton candy
Linalool	Floral woody	Floral
α-terpineol	Lilac, piney	Musty, wet dog, cilantro
carvone	Warm herbaceous	Floral, liquorice, mediceney
p-menthene-8-thiol	Grapefruity	rotten nutty grapefruit fruit
vanillin	Creamy, vanilla like	Vanilla
Nootkatone	Fruity, citrusy	Grapefruit

Arctander (1994). Nootkatone was described by panelists as rotten fruity, sweet grapefruity, stinky citrusy. Arctander's descriptor for nootkatone is citrusy. Comparison of retention times, indices and the odor description given by the panelists for the standard gives a good indication that same aroma active peak is being described.

Grapefruit Aroma

Nootkatone is considered by some scientists to be one of the important contributors to the grapefruit flavor (Stevens et al., 1970; Pino et al., 1986a; Shaw and Wilson, 1981). Maturity plays a significant role in determining the quantity of this sesquiterpene ketone. Traditionally, late season juices are considered to be best quality. Higher amounts of nootkatone were found in late (9.1 and 10.8 ppm) than in mid (3.2 and 3.9 ppm) and early (1.8 and 1.9 ppm) season unpasteurized and pasteurized juices. However, nootkatone was a poor predictor for juice quality (r=-0.05) in this 30 juice sample set of mid and late season juices.

Grapefruity aroma was also perceived by the panelists a few seconds before nootkatone has eluted. This peak had a Kovats indices or retention index (RI) of 1754. This peak has been tentatively identified as 8,9 didehydro nootkatone based on retention index and aroma quality. This has been reported to be present at 0.001 ppm level in grapefruit juice (Maarse and Visscher, 1989). Demole and Enggist (1986) reported its use to augment or enhance the organoleptic properties of grapefruit or imitation grapefruit beverages. This GC-O peak also occurs at the same time as one of the large sulfur peaks, RI-1753 (retention time 32 min). Since both these components have similar retention

times, it is not currently resolved which component is responsible for the additional grapefruit aroma peak. The question will have to be resolved with additional experiments using chromatographic columns of different selectivity. Another important sulfur component having a fresh grapefruity aroma is *p*-menthene-8-thiol. Discussion of this component is included in a later section.

Dilution Analysis

Studies involving dilution analysis (AEDA, Charm®) on grapefruit juice have not been reported to date. However, orange juice has been extensively studied (Marin et al., 1992; Hinterholzer and Schieberle, 1998) with both AEDA and Charm®. Among the components reported by the authors, hexenal, ethyl butyrate and vanillin were found to have highest dilution values, while linalool, decanal were found at the lower end of the dilution factors.

The peaks detected in our study were aroma active peaks from the juice extract concentrated 160 times. This does not provide information about which of these peaks have intense aroma activity at higher dilutions (lower concentrations). Since components in juice are not present in concentrated form, dilution analysis was done to identify the most aroma active, now referred to as aroma impact peaks. To assess the most intense peaks, juice extract was concentrated 16 times instead of 160 times and analyzed using GC-O as before. The list of peaks identified and their corresponding odors are given in Table 12. Some of the components like *cis* and *trans* linalool oxides were not present in the samples at 16 X concentration even though these components had intense aroma

activity (13 on a 15 point scale) in 160 X concentrated samples. da Silva et al. (1994) stated that odorants have different intensities above their threshold values, that is, aroma intensity may not be proportional to the concentration of the compound. According to Meilgaard et al. (1991), a mathematical model proposed by Beidler works best for middle and high range of sensory intensities. According to this model, there is a sigmoidal relationship between the concentration of the product and the stimulus perceived. This might be the reason for lack of odor perceptions of components like linalool oxides and linalool at lower concentrations of grapefruit juice aroma extract.

The two attributes which had intense aroma activity in the 16 X grapefruit juice extract were hexanal/ethylbutyrate and α-phellandrene (see Table 12). When these two components were used for sensory correlations (discussed in section 5 of results and discussion), they were found to have significant correlations (0.31 and -0.28 at $p< 0.05$) with aroma intensity. This suggests that hexanal/ethylbutyrate and α-phellandrene are key components in determining the quality of grapefruit juice.

Sulfur Compounds in Grapefruit

Detection

Organic sulfur compounds are present in a variety of food products and contribute significantly to their odor and flavor profile (Mistry et al., 1994). These are often present at sub-threshold levels and present a challenging task for chromatographers with respect to their detection. Mistry et al. (1994) compared a flame photometric detector (FPD), an

Table 12. List of components present in 16x concentrated juice extract and their intensities and aroma attributes

Components	Attribute	Aroma Intensities
Hexenal, Ethyl Butyrate	Green/Fruity	7.61
t-2-hexenal	Green	1.59
RI-863	Mushroom	3.12
RI-936	Sweet fruity	3.22
Sabinene	Unripe	3.06
Myrcene	Unripe citrusy	4.38
α-phellandrene	Green, Citrusy	7.20
para-cymene	Citrusy	5.26
trans-β-ocimene	Green, Citrusy	2.83
γ-terpinene	Floral, Citrusy	6.89
RI-1116	Grainy, Mediciny	3.02
allo-Ocimene	Sweet	4.89
RI-1141	Nutty	4.15
RI-1177	Green, Musty	3.33
α-terpineol	Musty	4.88
p-menthene-8-thiol	Stinky Grapefruit	4.76
RI-1349	Vinyl	6.39
RI-1374	Apple Sauce	3.76
RI-1381	Apple Sauce	6.03
Vanillin	Vanilla	4.91
RI-1464	Burnt	4.24
RI-1510	Perfumy	2.18
RI-1723	Incense	4.59
RI-1754	Grapefruity	3.08
Unknown Sulfur cmpd (RT 32min)	Grapefruity	2.29
Nootkatone	Grapefruity	6.76

atomic emission detector (AED) and a sulfur chemiluminescence detector (SCD). The authors reported best response in terms of sensitivity for AED. They rated FPD and SCD comparable to each other; however, FPD was not linear with the concentration of sulfur. SCD, on the other hand, had an equal molar response to all sulfur components.

The operation of sulfur chemiluminescence is based on the reaction of ozone with sulfur monoxide which is produced from combustion of analyte (Figure 16). The excited sulfur dioxide, upon collapse to the ground state, emits light with the maximum intensity of 350 nm. This detector is very specific for sulfur compounds, has equi-molar response and even the solvent peak was not detected.

Processing and Maturity Effects

The extraction solvent used for isolating sulfur compounds was ethyl acetate. This was found to extract more sulfur compounds than the solvent mixture of pentane-diethyl ether. The specific reason for this is not known yet. To our knowledge very little work has been done on sulfur compounds in citrus juices to make further comparisons and conclusions.

Twenty-two sulfur compounds were isolated in early, mid and late season pasteurized and unpasteurized grapefruit juice. This represents the most comprehensive determination of sulfur compounds in citrus juices reported to date. Total number and total sulfur peak areas decreased with increasing fruit maturity (Figure 17a) and increased with processing (Figure 17b). Total peak area of early season pasteurized juices was 83 times more than early season unpasteurized grapefruit juices. Late season pasteurized

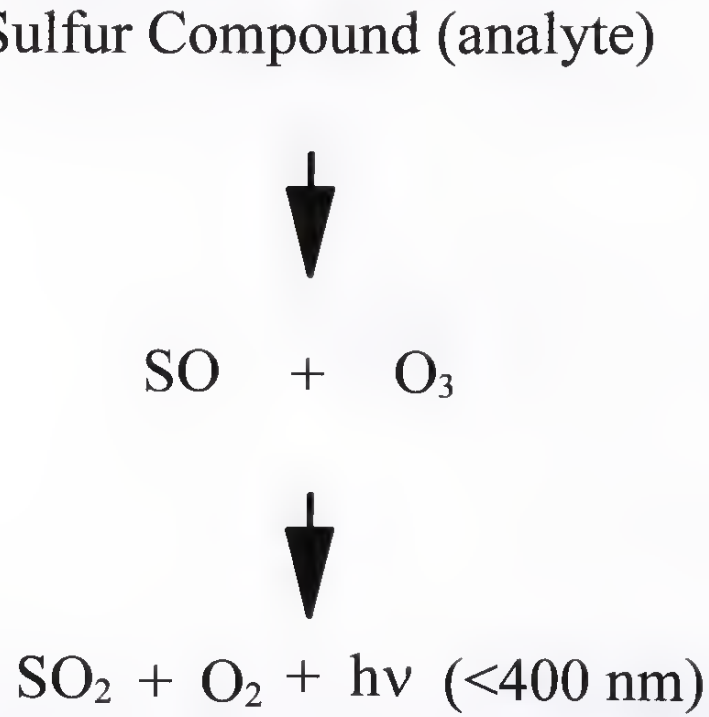


Figure 16. Sulfur chemiluminiscence reactions

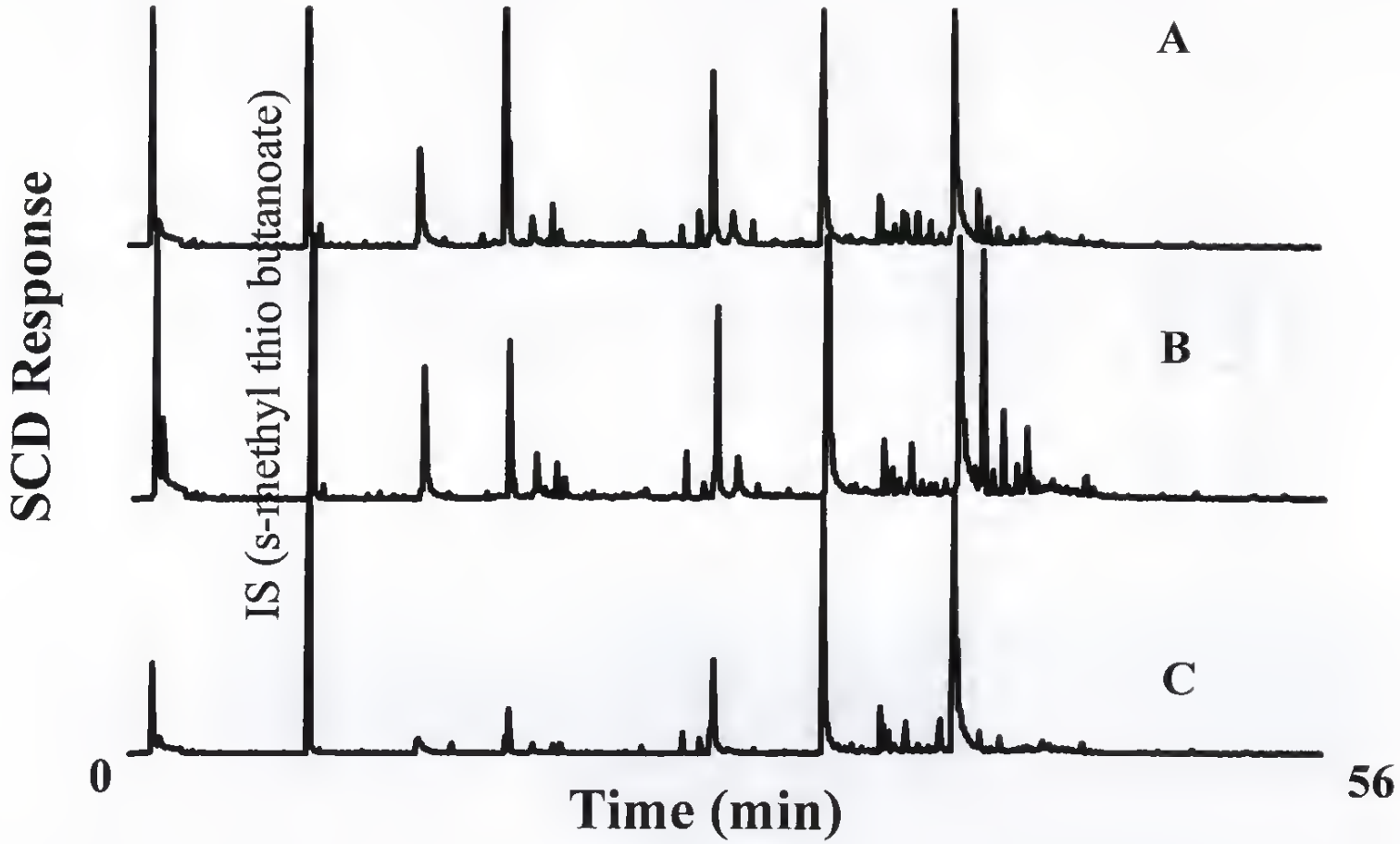


Figure 17a. Total number of sulfur peaks at different maturities in pasteurized grapefruit juice: A) early season, B) mid season, C) lateseason.

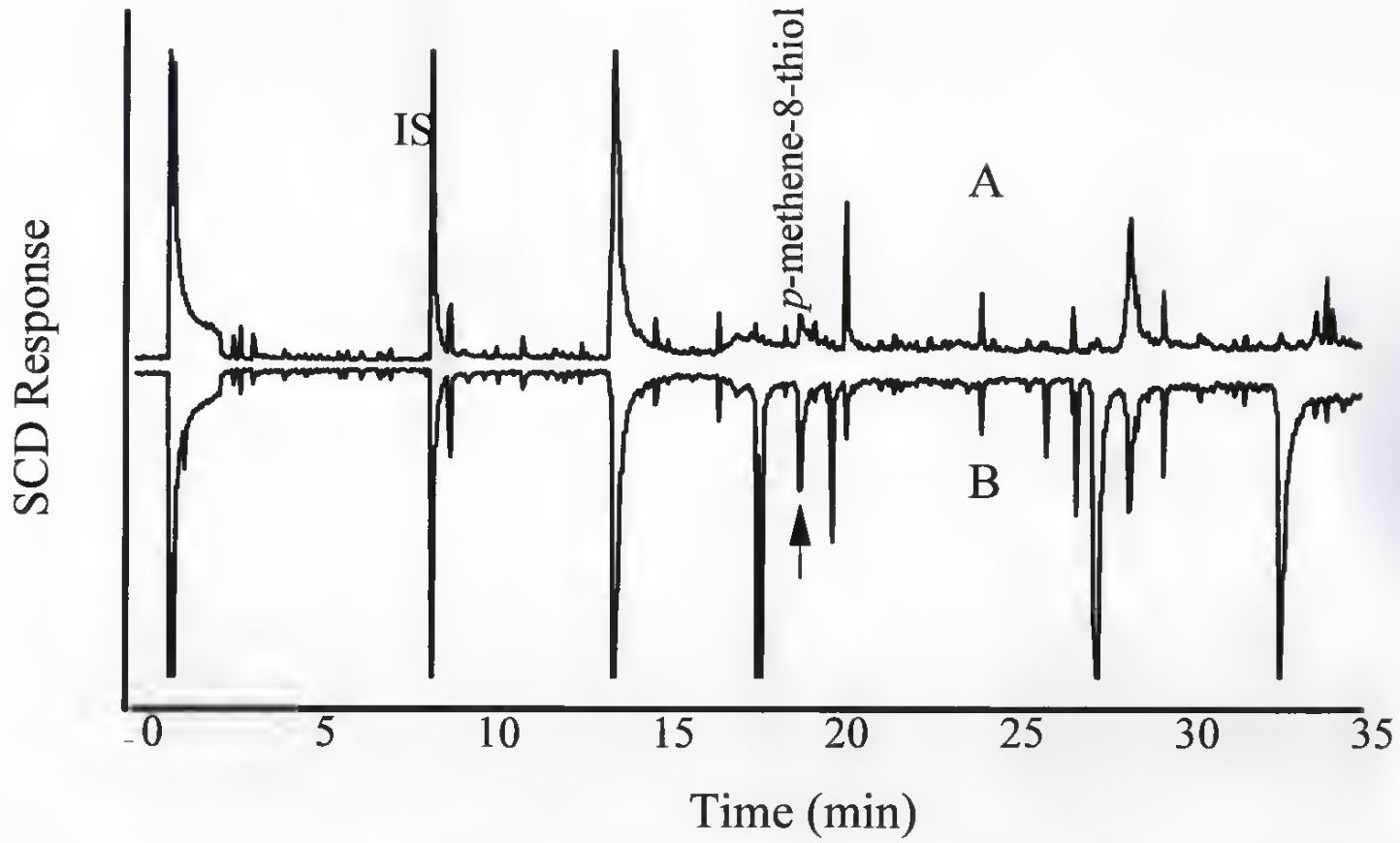


Figure 17b. Effect of pasteurization on sulfur compounds in early season grapefruit juice: A) unpasteurized and B) pasteurized

juices had 1.03 times more total peak area than the corresponding unpasteurized juices. We suspect that increase in tbese components might be due to thermal reactions occurring during pasteurization. Sulfur containing amino acids such as cysteine, glutathione (Miller and Rockland, 1952), and methionine (Burroughs, 1970) has been reported in fresb grapefruit juice. Thermal decomposition of these non-volatile sulfur containing amino acids might also be responsible for the increase in sulfur compounds due to thermal treatment.

Two of the SCD peaks bad the same retention times (20 and 32 min) as aroma active compounds detected in OSME. Both peaks were described as having "grapefruity" aroma by the panelists. One of the peaks, eluting at 32 min, bas the same retention index as didehydro nootkatone (RI-1754), which also has grapefruity aroma. Efforts to characterize this sulfur compound have not yet been successful. Therefore, tbe exact identity of this OSME peak could not be determined at this time.

p-menthene-8-thiol

The SCD peak eluting at 20 min has been identified as *p*-menthene-8-thiol. This was first reported by Demole et al. (1982) in grapefruit juice and bad a "fresh grapefruity" aroma. They found this thiol to have the lowest flavor threshold (10^{-7} ppb) in water of any substance yet reported. The level of this component in grapefruit juice was reported at 0.002 ppb by the authors. The concentration of this component was found at 4.6 ppb in early and late season pasteurized juice while the level in pasteurized mid-season juice was at 11.2 ppb. This terpene thiol was reported to be a reaction product of H_2S and limonene

(Demole et al., 1982) and the reaction is accelerated by higher temperatures. Since limonene is present at higher concentration in mid season juice, higher concentration of this component could be expected in this juice type.

A cyclization product of *p*-menthene-8-thiol, 2,8-epithio *cis-p*-menthane, was described by Demole et al. (1982) to have grapefruity aroma with a much higher odor threshold (9 ppb). The presence of this compound in our samples could not be established at this time, although our samples had a peak which closely matched with that of author's samples. The panelists did not detect any grapefruity aroma at the retention time of this compound (19 min), therefore this peak was not considered in this study.

Correlation Between Aroma Components and Sensory Measurements

The goal for this part of the study was to use the aroma active components (identified by GC-O) and taste components, and assess their contribution to the overall juice quality. For isolating the volatile aroma components, a more representative aroma extraction technique (pentane-diethyl ether) was employed.

Juice Classification

Juices were classified using natural clustering of average overall flavor scores of the 40 juices from the descriptive panel. There were three juices in the "worst" category. Average flavor scores were 6.3 or below. There were seven juices in the "fair" category. They had flavor scores between 7.31-7.83. Nine juices were classified as "good" and had

an average flavor score of 8-8.83. The 11 juices in the best category were rated above 9.12. In addition to rating the overall flavor for each juice, panelists evaluated grapefruit aroma & intensity, sweet/tart balance, and bitterness level.

Analysis of variance (STATISTICA 5.0) was conducted to judge the significance between the juice groups classified by the sensory scores. Duncan's multiple range test at 95% level was used. All the groups were significantly different from each other at $p<0.05$. The probabilities obtained in Duncan's multiple range post hoc tests with group as main effect are as follows: 0.0019, 0.000061, 0.000054 between worst and fair/good/best juices; 0.02, 0.000061 between fair and good/best juices; 0.00093 between good and best juices.

Sensory Analysis

Sensory attributes of a food product are a combination of odor, taste, visual impression and mouth feel. Volatile components contribute to the odor, while non-volatiles contribute to taste (sugars, acid etc.) and color (carotenoids, xanthophylls etc). Insoluble material contributes to the mouthfeel.

A descriptive taste panel was trained to quantify aroma attributes such as aroma intensity and aroma quality, and taste attributes such as bitterness and sweet/tart balance. The panelists were also asked to rate the overall flavor score using these attributes. In our earlier study preference scores were used to evaluate juice quality. The limitation of that approach was that it was not possible to determine why a particular juice was rated high

or low. A descriptive panel overcomes this problem by requiring the panelists to evaluate several flavor attributes in addition to overall flavor quality.

Table 13 shows the average minimum and maximum sensory scores produced by the panelists for grapefruit juice samples. As expected, grapefruit aroma intensity, quality and overall flavor score values were lowest in worst quality juices. The worst juices also had higher bitterness scores when compared to other juice types (see Table 13). However, the highest bitterness score for the worst juice types was only 8.66 on a 15 point scale. This can be attributed to the fact that this juice data set did not contain any early season juices. Since bitterness decreases rapidly with increasing fruit maturity, it is not surprising that the juice set contained few excessively bitter juices.

Sweet/tart balance scale ranged from -7 to +7 (see Figure 4). When the panelists perceived more tartness than sweetness, they rated the juices on negative scale. The value 0 represents equal balance between sweetness and tartness. All of the best juices were rated on the positive side ranging from 0.34 to 2.50. Thus all the best juices were slightly more sweet than sour. The range of sweet/tart values for all juice types can be seen in Table 13. There seems to be a trend in which higher ranked juices are considered more sweet than sour. Many of the worst juices also had higher bitterness score and lower grapefruit juice aroma intensity and aroma quality, which would have contributed to lower overall flavor score for these juices.

Table 13. Minimum and maximum descriptive sensory panel scores for grapefruit juices.

	Worst		Fair		Good		Best	
Attribute	Low	High	Low	High	Low	High	Low	High
GFT Intensity	6.29	8.69	7.37	8 43	6.67	10.08	6.72	9.10
GFT Quality	6.48	8.76	7.44	9.30	7.58	10.15	7.88	10.20
Balance	-0.12	0.89	-0.59	1.74	-0.27	1.74	0.34	2.50
Bitterness	4.00	8.66	3.10	6.62	3.29	7.59	3.28	6.53
Overall Flav.score	6.49	6.85	7.29	7.83	8.00	8 93	9.12	10.43

Univariate Analysis

Taste components

Univariate correlations of sensory data and taste components are shown in Table 14. Marked correlations are significant at $p < 0.05$. The sensory attributes evaluated here are: grapefruit aroma intensity, aroma quality, sweet/tart balance, bitterness and overall flavor score. The taste components which were evaluated include °Brix (sugars), acid (sourness), ratio of °Brix/acid (sweet/tart), limonin and naringin (bitterness).

The bitterness perceived by human subjects is a combination of two components, limonin and naringin. Since the taste threshold of naringin is 20 times greater than that of limonin (Guadagni et al., 1973), it can be assumed as a first approximation, that equibitter solutions of naringin and limonin would maintain this proportion.

When naringin and limonin were correlated individually with panel bitterness scores, the correlations were significant ($r = 0.85, 0.83$ respectively). Linear regression for limonin can be seen in Figure 18 and the corresponding plot for naringin is very similar. Rouseff et al. (1980) and Barros et al. (1983) observed similar behavior in earlier grapefruit juice studies. However, there was no significant correlations between limonin or naringin with bitterness in our earlier study employing a preference panel (Jella et al., 1998). To account for the total bitterness evaluated by the descriptive panel, limonin and 1/20th of naringin were combined and the correlation with bitterness score improved slightly ($r = 0.86$ at $p < 0.05$).

Table 14. Univariate correlations between sensory and taste components (Brix, acid, ratio, limonin and naringin).

	Aroma intensity	Aroma quality	Sweet/tart Balance	Bitterness	Overall flav.score	BRIX	Acid	Ratio	Limonin	Naringin	Limonin+ 1/20th of Naringin
Aroma intensity											
Aroma quality	0.75*										
Sweet/tart Balance	0.05	0.25									
Bitterness	0.34	0.05	-0.68*								
Overall flav.score	0.36*	0.54*	0.66*	-0.30							
BRIX	0.15	0.11	0.08	-0.19	-0.14						
Acid	-0.17	-0.44*	-0.22	0.13	-0.20	0.42*					
Ratio	0.27	0.50*	0.30	-0.32	0.05	0.47*	-0.61*				
Limonin	0.19	-0.11	-0.60*	0.85*	-0.27	-0.09	0.20	-0.31			
Naringin	0.22	-0.01	-0.58*	0.83*	-0.28	0.08	0.20	-0.16	0.88*		
Limonin+ 1/20th of Naringin	0.21	-0.05	-0.61*	0.86*	-0.28	0.01	0.21	-0.23	0.96*	0.98*	

* Correlations are significant at $p<0.05$

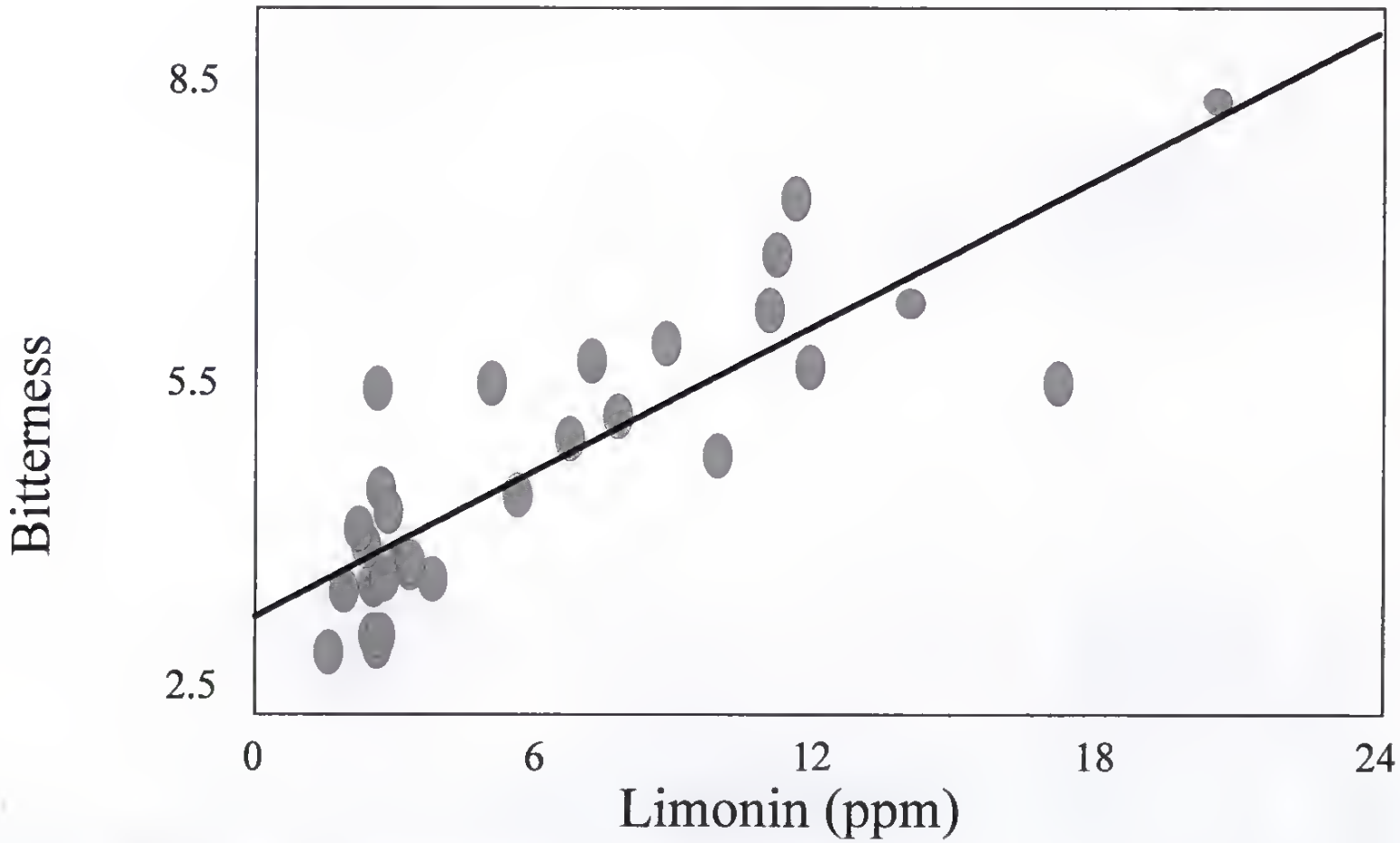


Figure 18. Correlation of limonin concentration with bitterness score

When these bitterness factors (naringin, limonin, and limonin + 1/20th naringin) were correlated with overall flavor score, the correlation was not significant at $p < 0.05$ ($r = 0.27, 0.28, 0.28$). Similar findings were reported by Pino and Cabrera (1988) and Jella et al., (1998). Low correlations between bitter components and overall flavor score might be due to the lack of early season, highly bitter juices in the sample set. As was noted earlier, the highest bitterness score recorded by the panel was only 8.66 out of 15. Thus, none of the juices were deemed to be excessively bitter. It should also be noted that some bitterness is expected and desired in grapefruit juice. Only when bitterness becomes excessive, are the overall flavor of the juices downgraded.

Brix (total sugars) and acid (titratable acid) are factors responsible for sweetness and sourness in grapefruit juice. The ratio between these two is important since it is one of the major quality standards used by the grapefruit industry. When panelists evaluated sweet/tart balance, it did not significantly correlate with Brix/acid ratio ($r = 0.30$ at $p < 0.05$). Analytical measurements of Brix and acid individually also did not correlate significantly with overall flavor score from the sensory panel ($r=-0.14$ and -0.2 respectively). However, as shown in Figure 19, when the sweet/tart balance sensory scores were compared with the overall flavor score rated by the panelists, there was a significant positive correlation ($r=0.66$). The reasons for differences in correlations within sensory (sweet/tart balance and overall flavor score) and between analytical vs sensory (ratio and sweet/tart balance) is explained below.

Perceived sweetness in citrus juices is due to sugars like sucrose, fructose and glucose which are 68-80% of total soluble solids. The analytical measurement, Brix, on

Figure 19. Correlation between overall flavor score and sweet /tart balance.

the other hand, measures not only sugars but other soluble solids present in the citrus juices nor does it differentiate between the relative sweetness of different sugars. Since panelists rate sweetness in juices based on the contribution of all sugars and not the total soluble solids, differences in the analytical and sensory measurements can be expected. Acidity (Sourness/tartness), as perceived by panelists, is due to free hydrogen ions in the juice, which is a direct measurement of pH. However, acid titrations with sodium hydroxide measures the total amount of available acid both free and undissociated. The acidity values reported in this study are titratable acidity rather than free acid, hence the analytical and sensory values in this study might be expected to have different correlations.

Grapefruit aroma quality and intensity are influenced by many components as discussed in section 3. Aroma intensity and aroma quality are affected by several factors such as maturity, processing history and juice extraction method. These two sensory attributes provide a first impression to consumers about the flavor quality of the juice. When grapefruit aroma intensity and quality were evaluated by the panel, the correlation with overall flavor score ($r = 0.36$ and 0.54 respectively) was significant at $p < 0.05$. These values suggest that aroma quality is more important than aroma strength in determination of overall juice flavor. Figure 20 shows the correlation between grapefruit aroma quality and overall flavor score. The correlation of aroma quality ($r=0.54$ at $p < 0.05$) is almost rated on a equal basis with that of sweet/tart balance and overall flavor score ($r=0.66$). This suggests that grapefruit aroma quality plays an important role in determining the overall quality of grapefruit juice. This is an important conclusion from

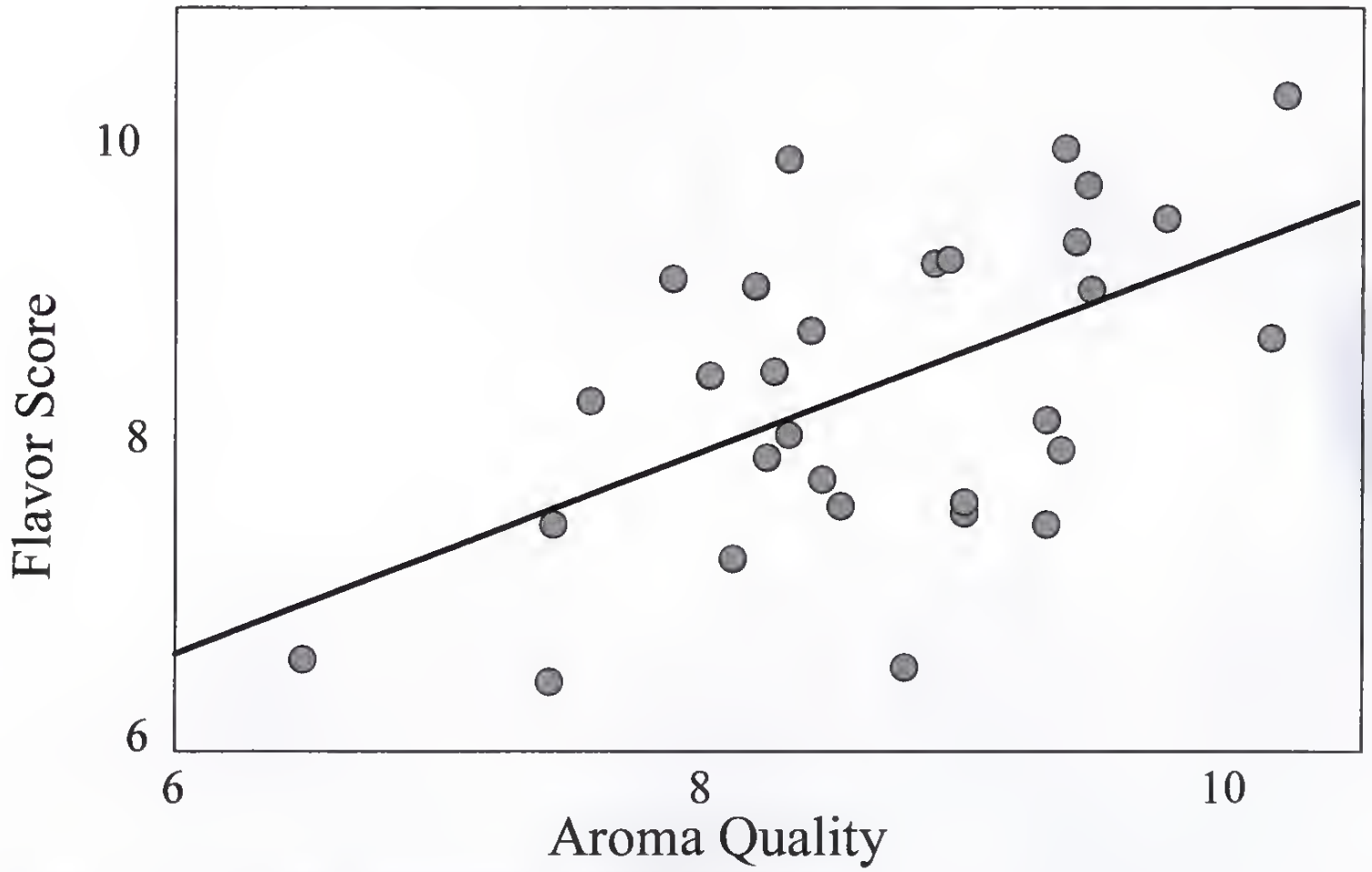


Figure 20. Correlation between aroma quality score and overall flavor score.

this study as the current industry standards are based solely on taste factors and no aroma components are considered.

Overall sensory quality judgements are made based on the interaction of many attributes. A balance between sensory components is required to create a positive overall sensory perception. The reason for non-significant sensory correlations with individual taste components might be due to a requirement of balance between several components. Panelists made additional comments when unusual flavor attributes were encountered. Comments for some of the lowest rated juices include: watery, bland mouth feel and off-flavor. The best juices were considered to have an optimum flavor balance.

Aroma components

Table 15 shows the univariate correlations between grapefruit aroma intensity, aroma quality, and overall flavor scores, and volatile aroma component FID peak areas. Myrcene, α-terpineol, vanillin, α-phellandrene, and several unidentified peaks were found to have significant negative correlations ($p < 0.05$) with aroma intensity scores. α-terpineol was reported to cause off-flavors in citrus juices (Clark et al., 1992). It tends to have a musty aroma at higher concentrations which might be objectionable to panelists. Hence it is negatively correlated with aroma intensity.

Hexenal and ethylbutyrate had significant positive correlations with aroma quality ($r=0.31$ and $r=0.31$ respectively) The other component having significant positive correlation with aroma quality is the peak at RI -1754. Hexenal and ethylbutyrate were described as having green and fruity smells in GC-O studies with a combined intensity of

Table 15. Univariate correlations between 26 aroma active volatiles and sensory scores.

Component	Correlation (r)		
	Aroma intensity	Aroma quality	Flavor score
Hexenal, Ethyl Butyrate	0.31*	0.31*	0.03
t-2-hexenal	-0.13	0.04	-0.19
RI-862	0.09	0.12	-0.08
RI-936	-0.56*	-0.42*	-0.33*
Sabinene	-0.17	-0.15	-0.14
Myrcene	-0.37*	-0.31*	-0.13
α-phellandrene	-0.28*	-0.20	-0.07
para-cymene	-0.16	-0.28*	0.08
trans-β-ocimene	-0.13	-0.11	-0.03
γ-terpinene	-0.09	0.00	-0.14
RI-1116	-0.24	-0.21	0.04
allo-Ocimene	-0.15	-0.16	-0.20
RI-1140	-0.25	-0.05	-0.14
RI-1177	-0.17	-0.07	-0.17
α-terpineol	-0.41*	-0.20	-0.29*
RI-1349	-0.11	0.18	-0.12
RI-1374	-0.22	0.05	-0.24
RI-1381	-0.28*	-0.07	-0.27*
RI-1464	-0.08	-0.09	-0.01
RI-1510	-0.21	-0.15	-0.23
RI-1722	0.15	0.14	-0.08
RI-1754	0.29*	0.31*	0.04
Nootkatone	0.02	-0.06	-0.05
p-menthene -8-thiol	0.08	0.00	0.11
Unknown Sulfur (32 min)	0.09	0.16	-0.31*
Vanilla	-0.37*	-0.25	-0.13

* Correlations are significant at $p<0.05$

7.61 on a scale of 15. The peak at RI - 1754 was described to have grapefruity aroma (intensity = 3.08). Since green/fruity and grapefruity attributes are perceived as positive qualities, these components can be expected to have significant positive correlations with the sensory scores. Pino et al. (1986a) also reported positive correlations of hexanal and ethylbutyrate with sensory scores.

Myrcene, *p*-cymene and an unknown compound were found to have negative correlations with aroma quality. The description given by the panelists for these components was green, unripe citrus smell of moderate intensity. The negative correlations with the sensory score (aroma quality) might be due to this green, unripe smell. It should be noted that myrcene was one of the three most differentiating components in our earlier study (Jella et al., 1998). Myrcene was highly negatively correlated with overall flavor. These more recent results suggest that it is also strongly negatively correlated with aroma quality.

When the aroma components were correlated with overall flavor score, α-terpineol and several unknowns exhibited negative correlations (Table 15). The effect of α-terpineol on sensory perception has been discussed in earlier sections. None of the most intense aroma components exhibited significant positive correlations with overall flavor score. Nootkatone, has been reported to have significant positive correlation with the sensory quality (Pino et al., 1986a). However, nootkatone failed to exhibit a significant correlation in our studies ($r = -0.05$ at $p < 0.05$). The correlations between aroma quality, intensity and nootkatone were also not significant. The lack of correlation between nootkatone and overall flavor score is clearly shown in Figure 21. Earlier studies

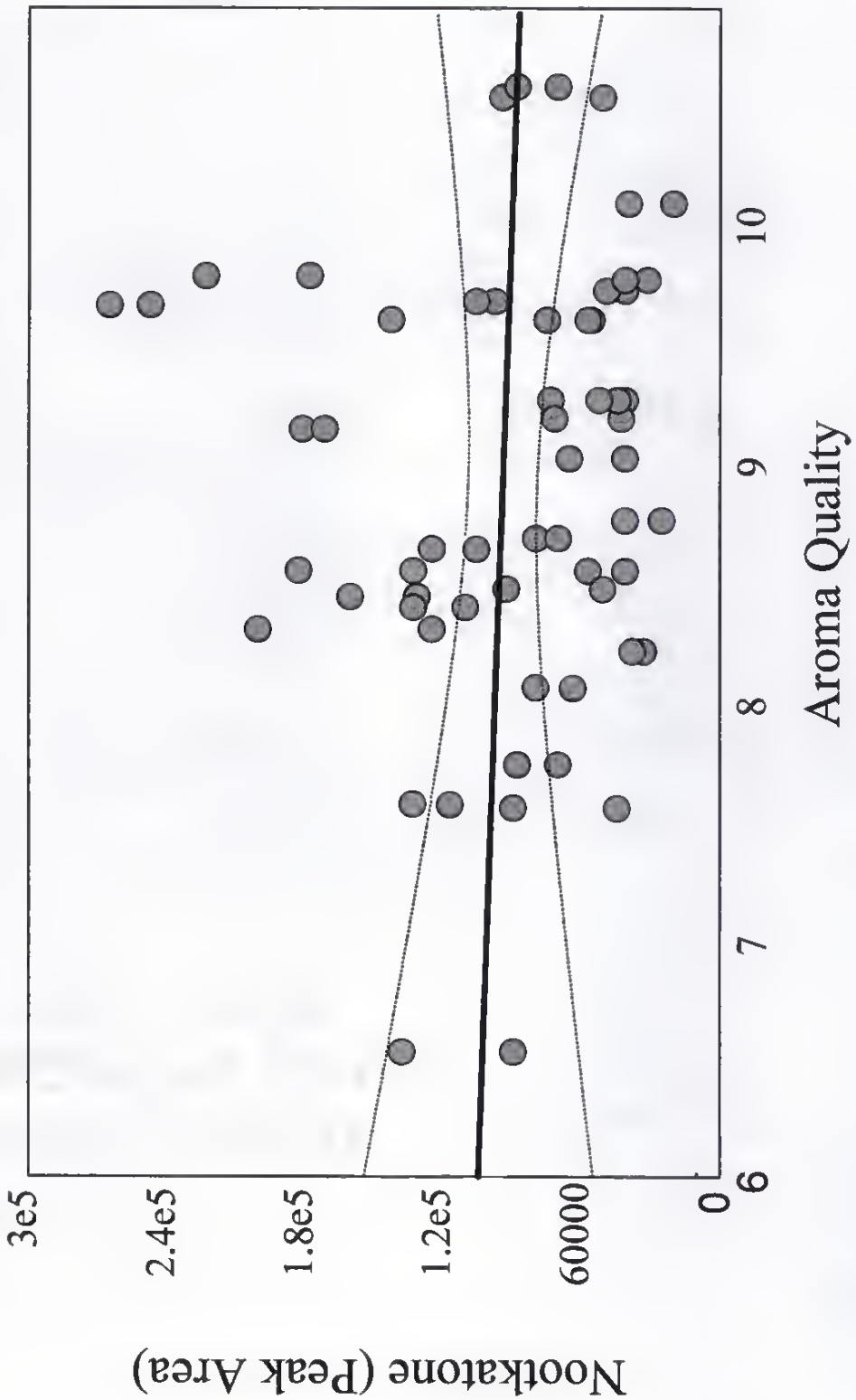


Figure 21. Correlation between aroma quality score and nootkatone peak area.

conducted in our lab also suggested a lack of significant correlation between nootkatone and sensory preference (Jella et al., 1998). Surprisingly, another important aroma component in grapefruit juice, *p*-menthene-8-thiol (Demole et al., 1982), also did not significantly correlate with overall juice quality ($r=0.08$ at $p < 0.05$). Therefore, components which have been suggested to be key factors for grapefruit flavor (nootkatone and *p*-menthene-8-thiol) had essentially no correlation individually with overall flavor.

Of the 26 most intense aroma volatiles, the component with retention index RI-936 exhibited significant correlations with aroma intensity (-0.56), aroma quality (-0.42) and overall flavor score (-0.33). This compound was described by the panelists as having sweet fruity aroma. When the retention index was compared with the literature value, the match suggested that the compound was α-thujene. However, the Arctander' descriptor for this compound was green, piney. Further conclusions regarding the exact identity of this compound could not be made at this time. However, in this study this compound seemed to be effective in differentiating the juices based on sensory quality.

Multivariate Statistical Analysis

Discriminant analysis can be used to determine which variables discriminate between two or more naturally occurring groups (Stat Soft, 1997). In this part of the study, juices were evaluated using values for the major taste components (limonin, naringin, Brix, acid and ratio), aroma active components and a combination of both taste and aroma components.

Twenty-six odor active peaks (24 from FID and two from SCD) chosen from GC-O dilution studies and five taste attributes (Brix, acid, ratio, limonin and naringin) were utilized for analyzing and predicting the juice quality using discriminant analysis. Of the 39 juices analyzed for aroma components, taste components and sensory attributes, data from 30 juices were used for the training set. Results of nine juices (chosen at random)were held back from training set and were used for testing the accuracy of the final flavor model.

Flavor models using taste components

None of the individual taste measurement compounds correlated significantly with the overall sensory scores (see Table 14). Therefore, multivariate statistical analysis was used to determine if a combination of taste components could be used to model and predict flavor quality. Taste components alone could not adequately discriminate the 30 juices in the training set. Whereas only 27% of the "good" juices, 50% of "fair" juices, and 68% of "Best" juices were successfully grouped, 100% of the "worst" juices were successfully grouped. This can be seen by the grouping of worst juices in the lower left hand side of Figure 22. This suggests that taste components best discriminate "worst" juice types.

When cumulative canonical coefficients (Root 1 and 2) of the taste components data were plotted in a 2D plot, positive Root 1 canonical coefficient scores were observed for all the worst juices, while the other three juice classes had negative coefficients (Figure 22). The first and second discriminant functions (root 1 & 2) are weighted most heavily by

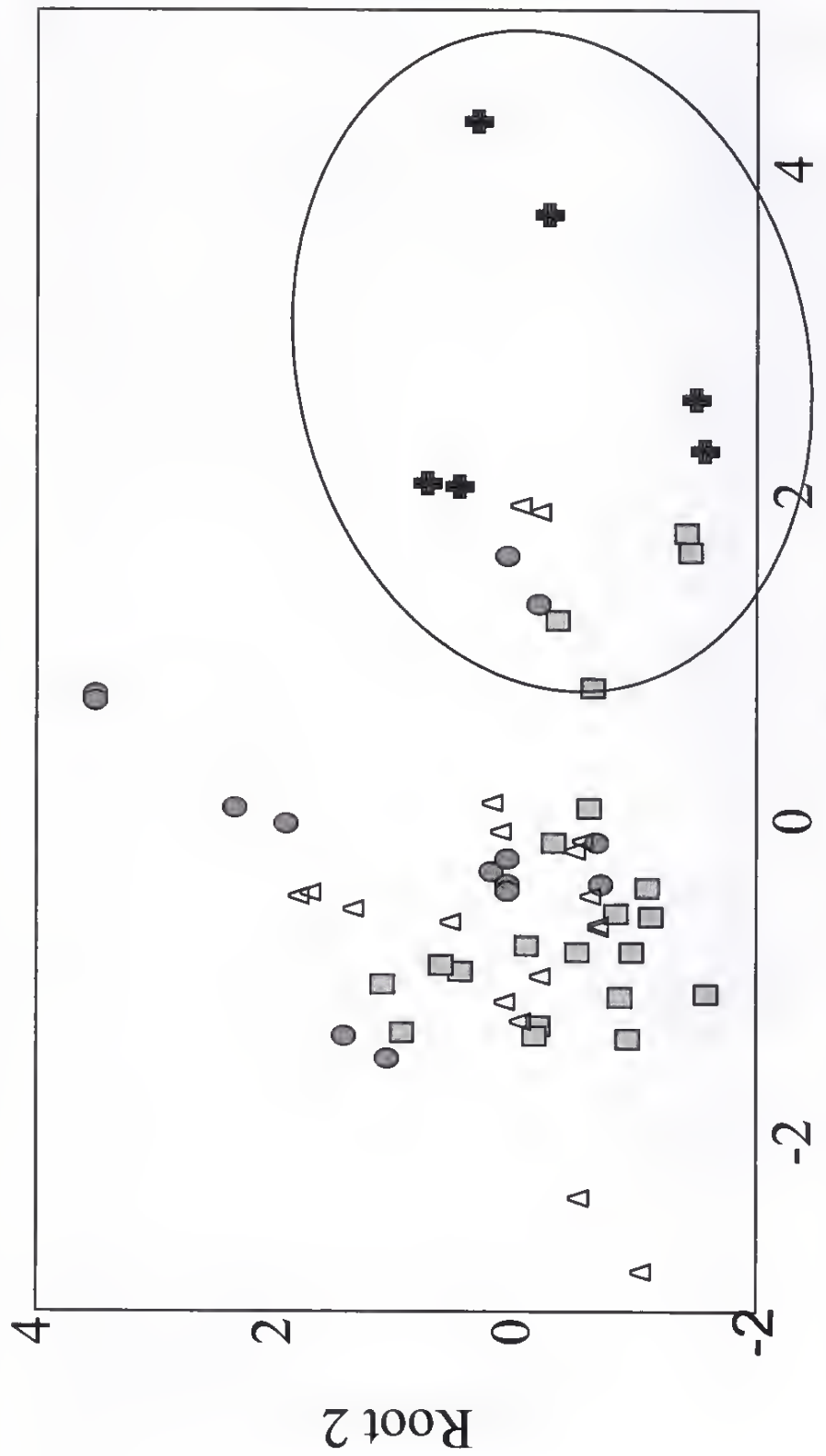


Figure 22. Standard discriminant analysis using 5 taste components: (✚) worst category, (●) fair category, (Δ) good category, (■) best category juices.

ratio and acid on the positive side (where worst juices are clustered) and by Brix on negative side (where good, best and fair juices are clustered). Their standard canonical coefficients were 10.1, 9.2, and -8.9 respectively. In comparison, the coefficients for naringin and limonin were surprisingly low (-0.7 and 1.2).

The squared Mahanalobis Distances (MD) is the distance computed between each case and the center of each group- "centroid". The larger the value between groups, the better they are discriminated. Comparing the group clusters in Figure 22 and also values in Table 16, it can be concluded that the 5 taste components only adequately separated the worst juices from other groups. Since MD value is a direct measure of how far apart the juices are from each other, lower values between fair, good and best juice classes (e.g.: distance between fair and best is 2.40) indicate that there is an overlap between them.

Data from nine of the 39 juices were used for testing the model. These nine juices were not included in the training set. Fifty-five percent of statistical classification agreed with actual sensory classification.

Flavor models using aroma components

Twenty-six volatile components (24 from FID and 2 from SCD) were selected based on their aroma activity for analysis. Based on their Kovats indices it was assumed that the 24 volatile components quantified from their FID peaks were the aroma active peaks identified by GC-olfactometry. However, some peaks may not be the aroma active peaks but another component that elutes from the GC at the same Kovat's index (retention time). It is known that some flavor impact components such as *p*-menthene-8-

Table 16. Squared mahalanobis distances for groups separated by taste components (Brix, acid, ratio, limonin, and naringin).

	Fair	Best	Worst	Good
Fair	0	2.396	10.222	1.592
Best	2.396	0	11.633	0.870
Worst	10.222	11.633	0	11.880
Good	1.592	0 870	11.880	0

thiol cannot be measured by FID or MS, hence the need for sulfur detector. Similarly there might be other potent components which can be detected only with specific detectors.

Earlier flavor models by Pino et al. (1986a, b), and Jella et al. (1998) have employed components that correlated highly with juice quality or acceptance. The components used in these models may or may not have had aroma activity. For example, β-caryophyllene was found to have high correlation with sensory preference (Jella et al., 1998). However, none of the sniff panelists perceived β-caryophyllene in the grapefruit juice extracts even though it exhibited a large FID peak. Thus, even though it was highly correlated, β-caryophyllene was not considered in the flavor model because no aroma activity was observed at concentrations found in grapefruit juice.

Standard discriminant analyses using aroma components. Standard discriminant analyses takes into consideration all the variables specified in the data set to build the model. The data set of 26 aroma active components had successfully grouped 100% of the 30 training juices classified as "worst", "fair" and "best". "Good" juices were 72% successfully grouped. When the mahalanobis distances for the good juices were calculated, the distances between good and best was least (11.5 units). The distance between good and fair was 21.4 units and between good and worst was 23.7 units. Lower the mahalanobis values, closer are the groups to each other. These values suggest there was some overlap between the "good" and "best" juices.

When the canonical coefficients were calculated, myrcene and RI-1510 had the largest negative root 3 canonical coefficient values (-2.9 and -2.5 respectively). When

canonical coefficients were plotted these components were effective in separating the worst juices from the other 3 juice classes. Univariate correlations also suggest that myrcene was significantly negatively correlated with sensory scores ($r=-0.37$ at $p < 0.05$). These findings about myrcene concurred with our earlier studies (Jella et al., 1998).

Forward step wise discriminant analysis using aroma components. To identify the most discriminating variables, forward step wise discriminant analysis was performed on the volatile data set (26 components). In this analysis, the program reviews all the variables, evaluates which one discriminates the most and includes that in the model. The program then proceeds to review the remaining variables, evaluates the second most discriminating variable, and includes that in the model. This procedure continues until all the components which help discriminate the data are included. In other words, the first component selected by the program is the most discriminating, second component is next best and so on.

When aroma active volatiles were analyzed with forward step wise discriminant analysis, 17 steps (tolerance level = 0.01 and F to enter = 1) were performed by the statistical program (i.e. these components were most efficient in separating the data set into 4 classes). The components which were selected (in order of most to least discrimination) are: RI-1381, Unknown sulfur (32 min), RI - 936, 1116, 1127, 858, Hexanal/ethylbutyrate, sabinene, RI -1464, *p*-cymene, nootkatone, α-terpineol, RI-1510, vanillin, trans-β-ocimene, myrcene and RI -1754. The component *p*-menthene-8-thiol considered by some as flavor impact component in grapefruit juice (Demole et al., 1982) was not in the list of discriminating components.

The above 17 compounds were utilized for grouping the training set data to see if statistical classification concurs with sensory classification. Best and fair juices were 100% successfully regrouped, while 83% and 72% of the worst and good juices were regrouped successfully. These results suggest that volatiles are best at differentiating the better juices and taste components best discriminate the worst juices.

Component RI -1381 and unknown sulfur compound eluting at (32 min) were most discriminating variables in the forward step wise discriminant analysis. When the peak areas of these two were plotted against each other, tight grouping of the best juices was observed. However, these two components by themselves were not effective in separating the other juice types

Flavor models using aroma and taste components

Grapefruit has a unique flavor which is a result of a combination of aroma components and a few taste components. In this part of the study, 5 taste components and 26 aroma active volatiles were selected for use in developing a flavor model.

Standard discriminant analysis. Data from the 26 aroma active volatiles and 5 taste components were used in testing the accuracy of sensory classification with statistical classification. In the training set, best, fair and worst juices were 100% successfully regrouped. Good juices were 94% correctly segregated. The mahalanobis distances for these four groups was highly significant (Table 17). The higher the values for mahalanobis distances, the farther apart are the juices. The distance between the best and the worst

Table 17. Squared mahalanobis distances for 26 aroma and 5 taste components (Standard Discriminant Analysis).

	Fair	Best	Worst	Good
Fair	0.000	36.391	60.356	38.847
Best	36.391	0.000	72.058	34.783
Worst	60.356	72.058	0.000	40.622
Good	38.847	34.783	40.622	0.000

juices was highest (72 units). This suggests that these two juice types were farthest from each other.

The mahalanobis values for good juices suggest that some of these were closer to the best and fair juices and hence it resulted in lower percentage (94%) for grouping good juice types. Mahalanobis distances between good and best juices was 34 units, between good and fair was 38 units, and between good and worst juices was 41 units.

The mahalanobis distances described above can be clearly pictured in Figure 23. This figure shows the cumulative canonical coefficients for different juice types using 26 volatiles plus 5 taste components. Root 1 was very efficient in separating best juices from worst juices. Best juices were on negative axis of Root 1, while worst juices were on positive scale. The ellipses around the juice types are at 95% confidence level. One outlier was observed in both best and good juice classes. Eighty-one percent of the variance was explained in Roots 1 and 2.

When canonical coefficients were calculated, myrcene weighed heavily on the positive side (7.74) in Root 1 where worst juices are clustered (see Figure 23). *trans* β-ocimene (-6.8) and nootkatone (-1.3) were on negative scale of Root 1 where best juices are grouped. Acid (17.43), Ratio (14.06) and Brix (-15.34) were instrumental in separating fair group from the other juice classes in Root 2.

Forward step wise discriminant analysis. Forward step wise discriminant analysis of the volatile and taste components was accomplished in 21 steps. There were 17 aroma

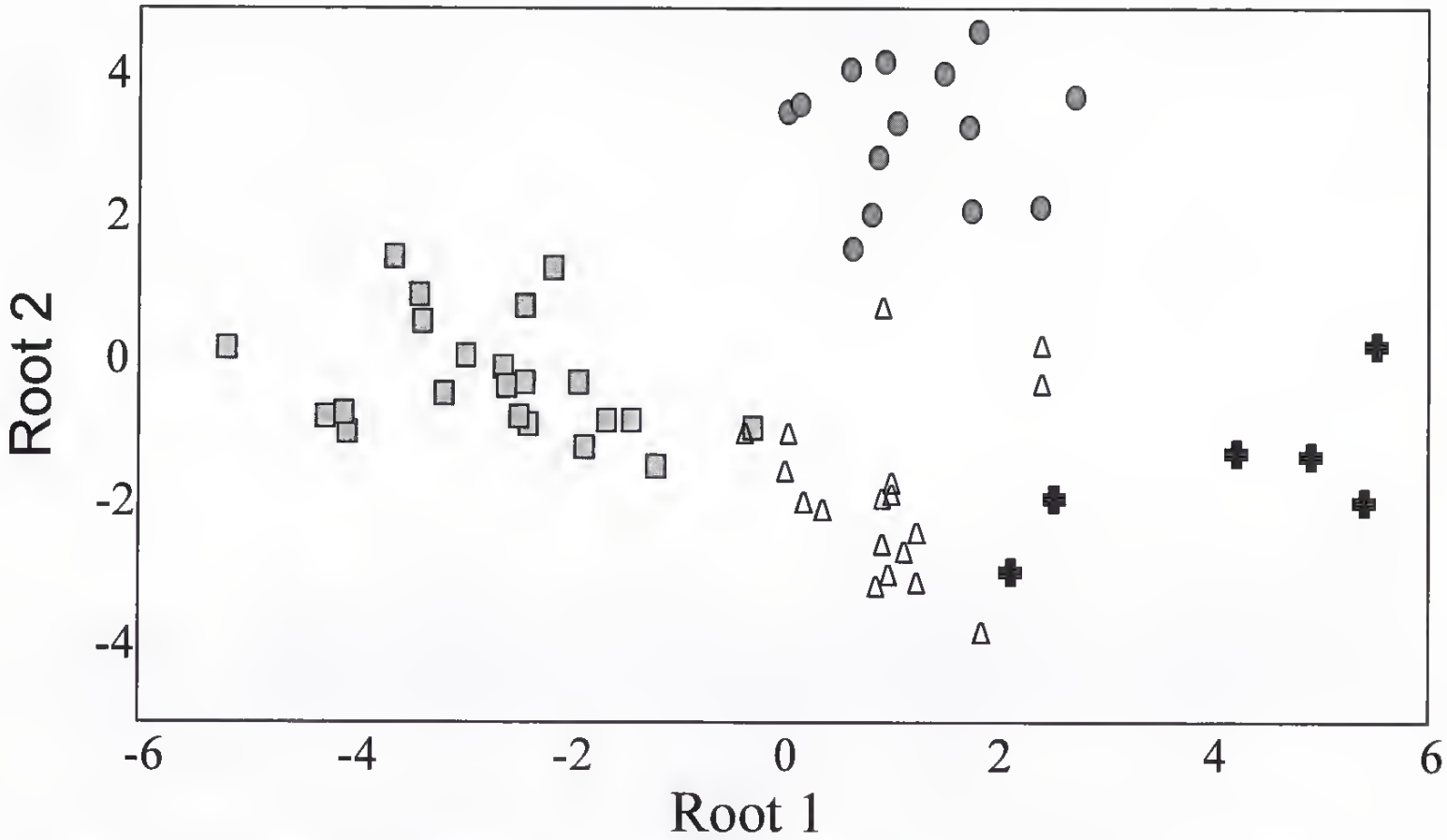


Figure 23. Forward stepwise discriminant analysis using 17 aroma and 4 taste components: (✚) worst category, (●) fair category, (Δ) good category, (■) best category juices.

components and 4 taste components. The volatile components selected, steps and Wilk's lambda values are given in the Table 18.

Wilk's lambda is a measure of the discriminatory power of a group of variables. The smaller the Wilk's lambda, the better the overall discrimination is. Perfect discrimination would occur at a Wilk's lambda of 0. Wilk's lambda value reported here represents the overall variance after the corresponding variable was included in the model. Since the discriminatory power of the function increases by adding a new variable, the corresponding overall Wilk's lambda value decreases. This is shown in Table 18.

When variables selected by step wise discriminant analysis (17 volatiles and 4 taste components) were analyzed and plotted, 100% separation of the juice classes was observed in training set. The groupings were similar to the ones observed in Figure 23. The model was tested by evaluating 9 juices not used in the training set. There were two replications for each juice (i.e. 18 samples). The flavor scores were known but the juices were treated as unknowns. Sixteen of 18 juices were correctly classified within one flavor category (Table 19).

To explain some of the mis classifications one must consider that some of the aroma active peaks detected in GC-O could be below the detectable range for analytical instruments. These components can be perceived by human nose since the sensitivity of it is much higher than analytical detectors. The FID peak area used in the model may not correspond to the aroma active component which occurs at the same retention time. Thus, quantitative values may not reflect actual concentrations of the aroma active component and therefore reduces the effectiveness of the model. However, it should be

Table 18. Forward step wise discriminant analysis-volatiles and taste components (Number of steps and corresponding component).

Component	Step	Wilk's lambda
Acid	1	0.642
RI-1374	2	0.456
Unknown Sulfur(32 min)	3	0.340
RI-1127	4	0.250
RI-1510	5	0.210
α-terpineol	6	0.178
Limonin	7	0.145
RI-1116	8	0.121
RI-1464	9	0.104
p-cymene	10	0.082
Brix	11	0.065
Ratio	12	0.050
RI-1754	13	0.040
RI-1141	14	0.035
RI-863	15	0.026
RI-1723	16	0.022
Nootkatone	17	0.017
RI-1381	18	0.015
RI-985	19	0.014
Myrcene	20	0.013
RI-1047	21	0.011

Table 19. Comparison of sensory and statistical classification of grapefruit juices. (Model has been tested using 17 aroma components and 5 taste components)

Juice		Classification	
Batch	Replication	Sensory	Statistical
A	1	Best	Good
A	2	Best	Good
B	1	Good	Good
B	2	Good	Good
C	1	Good	Best
C	2	Good	Fair
D	1	Best	Best
D	2	Best	Best
E	1	Fair	Fair
E	2	Fair	Fair
F	1	Best	Best
F	2	Best	Best
G	1	Best	Best
G	2	Best	Best
H	1	Good	Good
H	2	Good	Good
I	1	Worst	Good
I	2	Worst	Good

emphasized that while the model is not 100% accurate it is quite good at predicting flavor quality. It represents the most accurate flavor model developed to date for grapefruit juice.

CHAPTER 5
CONCLUSIONS

Aroma and taste are the predominant factors influencing the flavor of grapefruit juice. One of the goals of this study was to identify the key aroma impact volatile components in grapefruit juice. These aroma active volatile components, along with non-volatile taste components, were utilized to accurately predict grapefruit juice quality.

Correlation Between Preference and Analytical Measurements

Multivariate statistics helped identify which analytical measurements best correlated with sensory preference measurements. Nootkatone was effective in accounting for the variance in the total data set but relatively ineffective in differentiating between juices of various flavor preference. Myrcene, β-caryophyllene and linalool could be used to differentiate juices of various flavor preference. Using discriminant analysis they could correctly predict preference category for 74 % of the samples. At least 19 components were required to correctly predict the preference category for 100% of the samples using forward step wise discriminant analysis. However, using backward step wise discriminant analysis, it was possible to construct a predictive model using only 16 components. It should be emphasized that the models developed to predict flavor preference are based on statistical correlation only and may or may not be causative.

These models may not include all the flavor impact compounds, as it is not necessary to have a statistical model which includes all (or any) causative components as long as other components consistently correlate.

Taste components were included in almost all predictive models despite the fact that there were 10 times as many volatile components measured. This strongly suggests that taste is an important component in any grapefruit juice flavor model. However, there were no successful models which contained only taste components. Therefore, the most successful models must contain hoth taste (non volatile) and aroma (volatile) components.

Aroma Extraction Methods

Isolation of flavor volatiles is one of the most important and limiting step in food aroma analysis. There is no single extraction method which can extract all the aroma components in the exact proportion they exist in the sample. Each procedure will concentrate some components and to varying degrees discriminate against others. Three methods of extracting the flavor volatiles were compared. Liquid-liquid, (pentane:diethyl ether-1:1), static head space (solid phase micro extraction-SPME), and dynamic head space analysis (Tenax/charcoal trap) with solvent elution were used to extract volatiles from a single juice.

Pentane-diethyl ether extracts produced chromatograms with the best balance of top notes, terpenes and sesquiterpenes. However, it also extracted undesirable high hoiling compounds which increased GC analysis time. When compared with methylene

chloride liquid-liquid extraction, pentane-diethyl ether solvent extraction gave 73 % more top note peak area.

Dynamic head space extracted primarily top note volatiles. The top note area was 160 % times more than pentane-diethyl ether liquid-liquid extractions and 350 % more than methylene chloride liquid-liquid extractions. However, few of the higher boiling compounds could not be analyzed using dynamic head space analysis.

Static head space-SPME extracts contained relatively small amounts of top note volatiles compared to other extraction methods. It preferentially concentrated terpenes. The main advantage of this is that it is simple, rapid does not use an extracting solvent which would obscure highly volatile compounds. However, it exhibited the poorest analytical reproducibility.

Of the above three extraction methods evaluated, pentane-diethyl ether extracts gave the most representative chromatographic profile. Hence, this procedure was chosen to complete the remainder of the study.

GC-Olfactometry

Maturity and heat treatment effects were determined using pasteurized and unpasteurized (early, mid and late season) juices. Of the 150 components separated from grapefruit juice aroma extracts, 80 were identified and are listed in Appendix D. Of these 80 components, approximately 37-49 exhibited aroma activity as identified by GC-olfactometry. There were 25 aroma active components with intensities high enough that

they should be considered key aroma impact components. Increased fruity attributes were observed in late season juices when compared to early season juices. Several aroma components were lost due to processing. Earthy, skunky and liquorice type odors were found in pasteurized but not in unpasteurized juices. 1-*p*-menthene-8-thiol, a sulfur character impact compound produced an intense aroma response but was not detected using FID. A strong vanilla aroma peak was detected in all the juices. It has been identified as vanillin and is reported for the first time in grapefruit juice.

Sulfur Compounds in Grapefruit Juice

A routine method for grapefruit juice sulfur compounds employing ethyl acetate extraction and sulfur chemiluminescence detection was developed for the first time. Using this process, 22 compounds were quantified in early, mid and late season pasteurized and unpasteurized grapefruit juices. This represents the most comprehensive determination of sulfur compounds in citrus juices reported to date. Previous procedures were limited to low molecular weight sulfur compounds such as hydrogen sulfide or dimethyl sulfide. Total number and total sulfur peak areas decreased with increasing fruit maturity. Thermal processing increased the sulfur peak areas. Peak area in early season pasteurized juices was 83 times more than the early season unpasteurized grapefruit juices. Late season pasteurized juices had 1.03 times more peak area than corresponding unpasteurized juices. The reason for this increase in peak area might be due to reactions occurring at high temperatures and also degradation of non-volatile sulfur components such as cysteine

and glutathione. At least two SCD peaks bad the same retention times as aroma active compounds detected using gas-chromatography olfactometry (OSME). One of tbese peaks, *p*-menthene-8-thiol, produced a small SCD peak, but an intense OSME peak.

Correlations Between Aroma Components and Sensory Measurements

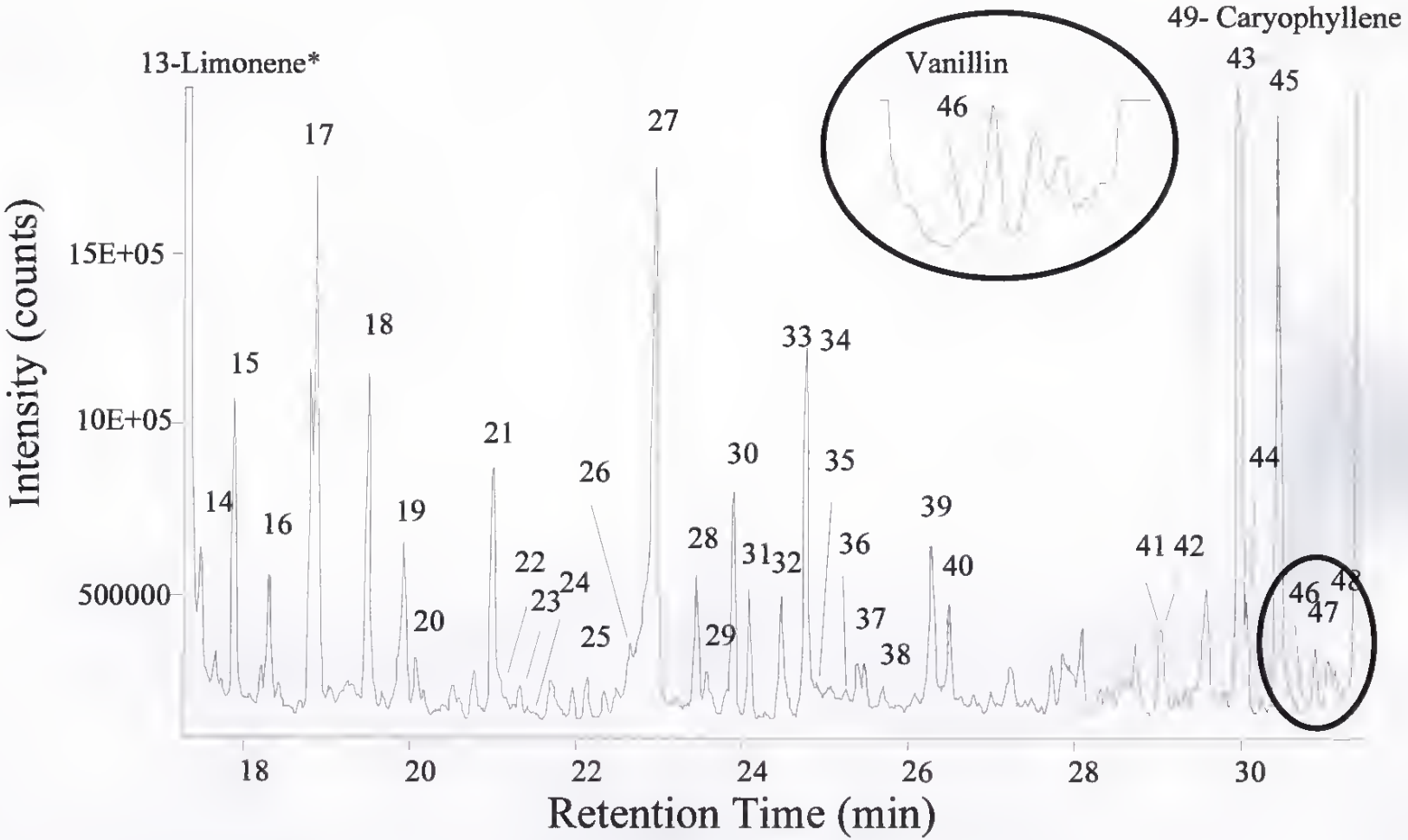
The relative correlation of 26 aroma impact components extracted by pentane-dietbyl ether and identified by GC-O, and 5 taste components with overall sensory scores were determined. The worst quality juices had the lowest sensory scores for grapefruit aroma intensity and quality (6.29 and 6.48 respectively). Significant correlations were observed between bitter limonin/naringin concentrations and bitterness score ($r=0.85$ and 0.83 respectively at $p < 0.05$). However, there were no correlations between these bitter components and overall flavor score. Tbe best juices were rated as being more sweet than sour on sweet/tart balance scale (see Table 14). Wben correlated witb overall sensory score, botb grapefruit aroma quality and sweet/tart balance had relatively equal significant values ($r=0.54$ and 0.66 at $p < 0.05$). This indicates that the contribution of aroma to tbe overall flavor perception is as important as taste components. This is an important conclusion from this study as many of the previous studies and the current industry standards are based solely on taste components. When sweet/balance sensory scores were correlated witb the corresponding analytical value (Ratio), the correlation was not significant.

Among the volatile components, myrcene, α-terpineol and several unidentified peaks were found to have significant negative correlations (see Table 15) with aroma intensity and quality. Hexanal, ethylbutyrate were found to correlate positively with aroma quality. Nootkatone was not a significant factor in univariate analysis, but in multivariate analysis along with trans-β-ocimene and several unknown compounds, it was effective in discriminating the best juices.

Discriminant analysis was used to classify the juices based on sensory scores. Data from taste components was efficient in differentiating the worst juice class. Tight clustering of best juice types was observed when volatiles were used to separate the juice types. This suggests that taste components are important in separating worst juice types and volatile components for the best juice types.

Finally 17 aroma active volatiles and 4 taste components were successfully employed in a grapefruit juice flavor model based only on components with flavor activity. The model could separate training juices with 100% accuracy. This model was further tested by evaluating nine juices (2 replications each) not used in the training set. The flavor scores were known but the samples were treated as unknowns. Sixteen of the 18 samples were correctly classified within one flavor category. This model represents the most accurate and comprehensive flavor model developed to date. It could be used by the grapefruit processing industry to improve both flavor consistency and quality.

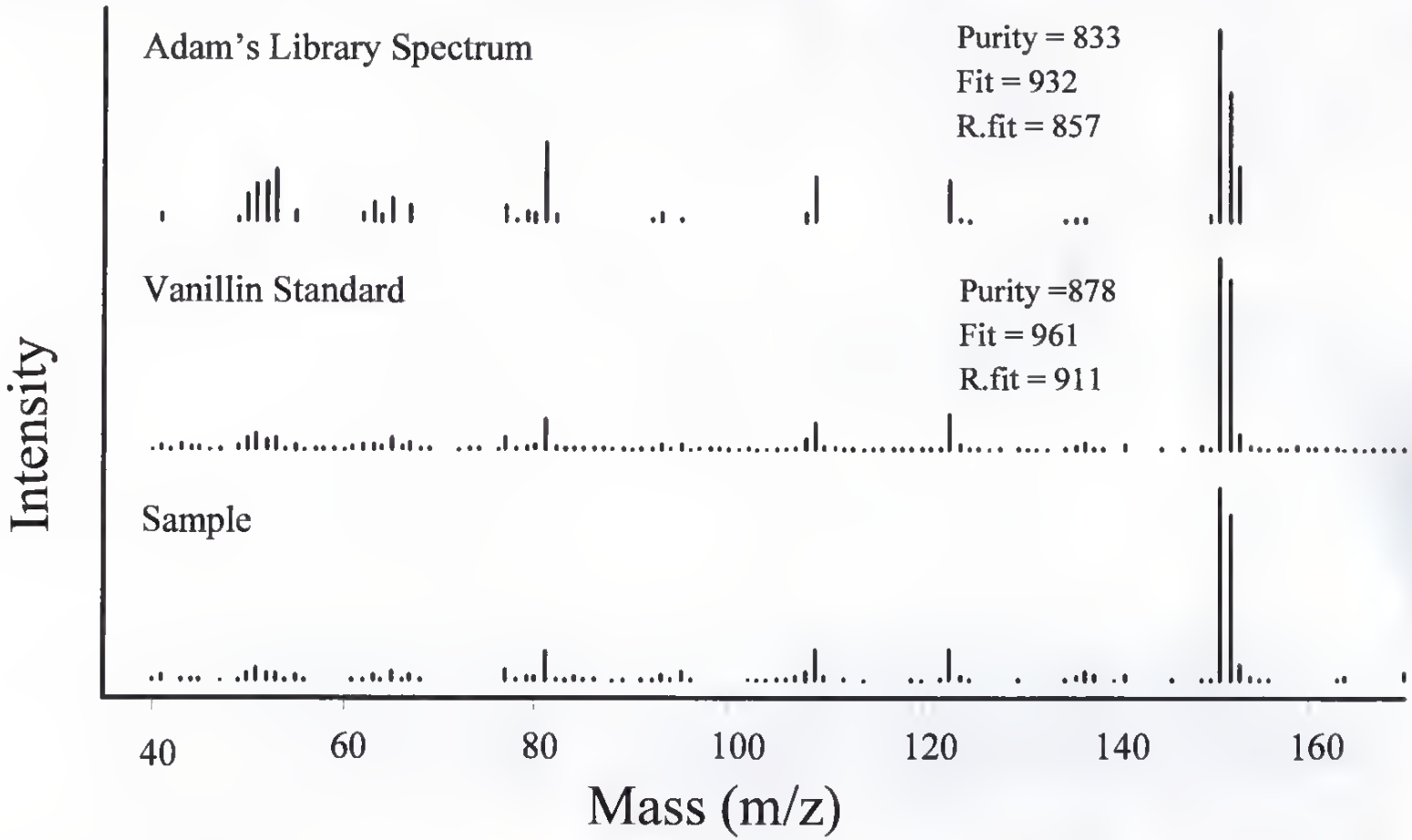
APPENDIX A
TOTAL ION CHROMATOGRAM OF LATE SEASON GRAPEFRUIT JUICE



Appendix A. Total ion chromatogram of late season grapefruit juice highlighting vanillin peak

* Components Identified in Appendix D

APPENDIX B
MASS SPECTRUM OF VANILLIN



Appendix B. Comparison of mass spectrum of vanillin (from grapefruit juice extract) with library and reference spectra

APPENDIX C
LIST OF DESCRIPTORS AND THEIR RELATIVE INTENSITIES (GC-O)

Appendix C. Average attributes perceived by the panelists in juice extracts of different maturities.

Kovats	Aroma intensities rated by panelists (GC-O)						Attributes
	Early Unp.	Early Past.	Mid Unp.	Mid Past.	Late Unp.	Late Past.	
722	9.0	7.8	7.1	9.2	8.6	9.7	Fruity
728	7.6	5.4					Fruity
801	9.0	8.8	8.9	7.7	9.3	10.2	Green
812			9.6	9.1	10.7	10.8	Chemical Stink
817	9.6	8.3					Green, dead bug
842	6.4	6.5	5.8	6.8	7.9	6.1	Fruity
850						10.3	Roasted Grain
862	9.6	7.4	8.9	11.6	11.1	11.2	Oatmeal
882	7.0			4.0			Citrus
885	7.9	7.3	9.1	6.9	10.1	9.5	Cooked Oat
901		5.7			7.3	7.9	Floral
934	8.2		8.5	7.6	10.4	11.9	Vitamin C, Cat Urine
965*	13.7	12.4	14.2	14.8	13.8	13.7	Cherry, Almond
981	10.4	12.0	9.9	10.8	11.2	11.0	Unripe Mango
984	12.0	7.1	10.2	11.6	10.8		Sweet Fruity
989	6.5	11.3	8.5	9.1	9.7	6.8	Minty
997				6.9	12.3	11.4	Citrus Lemony
1003	12.3		11.0	12.7		12.4	Citrus, Lemon Grass
1026			7.7	8.3	6.9	7.4	Minty
1030	9.9	6.8					Citrusy
1040	7.6			5.8			Citrusy
1047	9.5	6.2		7.0	11.8	7.8	Floral
1050			5.5	8.5			Unripe Cucumber
1060	10.2	10.0	8.6	11.5	12.1	14.0	Cotton candy
1072			9.8	6.6	6.3	6.0	Burnt Sugar
1089	12.0	12.0	11.3	13.7	13.5	13.9	Cotton candy Citrusy
1095					11.3		Cotton Candy
1098	10.3		12.6	12.1	13.1	10.6	Burnt Sugar, fruity
1101	11.3	10.0	11.3	12.0	12.4	12.4	floral
1112	12.0	10.1	12.0	8.9		8.8	Cooked Oat
1120	12.7		9.8	11.3	10.2	8.8	Cooked Rice, Mediciny
1130	11.1	9.8	10.6	9.0	11.5	9.8	Terpeney
1148					9.3		Greenish Floral
1165	10.4		9.9	13.1	11.1	11.8	Rubber, Vinyl
1176	7.4	8.6	5.9	7.2	7.8	8.2	Terpeney

Unp = Unpasteurized,
Past. = Pasteurized.

Appendix C. Continued..

Kovats	Aroma intensities rated by panelists (GC-O)						Attribute
	Early Unp	Early Past.	Mid Unp	Mid Past	Late Unp	Late Past.	
1187				7.5			Alcohol, Distelleri
1201	11.2	8.9	10.6	12.6	9.3	9.9	Cilantro
1225		7.3	7.2	6.8	10.4	10.7	Dead Bug Terpeney
1230		9.3			7.8		Earthy, Musty Cooked
1251		7.8				7.0	stinky feet, stinky fruit
1269	7.9	6.9		4.0			Floral
1272			8.0	6.9			Savory
1299	8.6	8.7	9.1	9.1	9.5	8.6	p-menthene thiol
1312		10.8		10.3	8.5		Rancidoil
1318	12.2	10.8	10.3	11.7			Spicey Oily
1350			8.0	10 8	10 5	13.0	Coal Smokey
1360			5.0	7 8			Greenish, Vinyl
1375	6.8		7.1	10.2		5.6	Fruiy, Peach apricot
1384	9.2			10.4	9.9	9.2	Cooked Caramalized
1404	14.6	11.8	12.5	14.0	10.5	12.9	Vanilla
1440						4 6	Fruity, watery Fumes
1468					12.6	7.0	Musty
1492	9.1	6.1	9.0	11.0	11.2	10.4	Floral
1509		7.2	6.7	8 6			floral, fresh
1638*	13.2	5.8	11 4	13.5	13.0	13.2	Jasmine
1659			5.3				Mushroomy
1665		4 0	7.4		8 8		peppery
1670			7.0				Stinky Bad Breath
1680	7.2					6.7	Pungent
1690	9.8	5.8					Stinky Gft
1708	6.3	7.4	5.7	10.0		10.4	peppery
1717			8.7	8.5			Pepper
1722	8.6			7.2	7.1	10.2	Rotten GFT
1730					6.6		Stinky GFT, Citrusy
1740					10.3		Grapefruit peel Oil
1754	10.0	6.2	6.7	7.3	11.9	13 1	Floral
1790	8.5	8 9	13 4	10.8	12.5	11 0	GFt
Total Peaks	41	37	43	49	43	43	

* Internal standards.

APPENDIX D
COMPOUNDS IDENTIFIED IN NOT-FROM-CONCENTRATE GRAPEFRUIT JUICE

Appendix D. Compounds identified in not-from-concentrate grapefruit juice.*

No.	Component name	Retention Index (Adams R P. 1995)	Aldehydes	Esters	Alcohols	Ketones	Hydrocarbon
1	Propyl Acetate			+			
2	Hexanal	800	+				
3	Ethyl Butyrate	800		+			
4	Ethyl Acetate	807		+			
5	Furfural	830	+				
6	α-Thujene	931					+
7	Thuja-2,4(10)-Diene	956					+
8	Benzaldehyde (IS)	961	+				
9	Myrcene	991					+
10	Ethyl hexanoate	996		+			
11	α-Phellandrene	1005					+
12	α-Terpinene	1018					+
13	Limonene	1031					+
14	*cis*-β-ocimene	1040					+
15	*trans*-β-Ocimene	1050					+
16	γ-Terpinene	1062					0
17	*cis*-linalool oxide	1074					
18	*trans*-linalool oxide	1088					
19	Linalool	1098			+		
20	Nonanal	1098	+				
21	Iso-propyl hexanoate			+			
22	*allo*-ocimene	1129					+
23	*cis*-limonene oxide	1134					
24	*trans*-p-menth-2-en-1ol				+		
25	β-Pinene oxide	1156					

*private communications - Kevin L. Goodner.

Appendix D. Continued..

No.	Component name	Retention Index (Adams R P. 1995)	Aldehydes	Esters	Alcohols	Ketones	Hydrocarbon
26	Nonanol	1171			+		
27	Terpin-4-ol	1177			+		
28	α-Terpineol	1189			+		
29	Ethyl Octanoate	1195		+			
30	Decanal	1204	+				
31	Dihydro citronellol	1196			+		
32	*trans*-carveol	1217			+		
33	Nerol	1228			+		
34	Citronellol	1228			+		
35	*cis*-carveol	1229			+		
36	Neral	1240	+				
37	Carvone	1242				+	
38	Geraniol	1255			+		
39	Geranial	1270	+				
40	Perilla aldehyde	1271	+				
41	α-Terpinyl acetate	1350		+			
42	α-Cubebene	1351					+
43	α-Copane	1376					+
44	Geranyl acetate	1383		+			
45	β-Cubebene	1390					+
46	Vanillin	1391	+		+		
47	Decanol acetate	1409		+			
48	α-Cedrene	1409					+
49	Caryophellene	1418					+

Appendix D. Continued..

No.	Component name	Retention Index (Adams R.P. 1995)	Aldehydes	Esters	Alcohols	Ketones	Hydrocarbon
50	β-Gurjunene	1432					+
51	α-Guaiene	1439					+
52	Geranyl acetone	1453				+	
53	α-Humulene	1454					+
54	β-Cadinene	1473					+
55	germacrene	1480					+
56	δ-Cadinene	1524					+
57	Caryophyllene oxide	1581					
58	Methyl jasmonate (IS)	1647		+		+	
59	Aristolone	1767				+	
60	Nootkatone	1800				+	

LIST OF REFERENCES

Acree, T.E.; Barnard, J.; Cunningham, D.G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273-286.

Acree, T. E.; King, K. M. Identification of aroma-impact compounds in three Japanese teas. *Institute of Food Technologists Annual meeting.* **1996**, *147*, 1082-1236.

Adams, R. P. Ion trap mass spectra. In *Identification of essential oil components by gas chromatography / mass spectroscopy*; Allured Publishers: Carol Stream, IL, 1995; pp 45-449.

Aiken, J. W.; Noble, A. C. Comparison of the aromas of oak- and glass-aged wines. *American Journal of Enology and Viticulture* **1984**, *35*, 196-199.

Akieda, T.; Kato, T. [Analysis of aroma components in coffee flavour.]. *Reports of the Central Customs Laboratory [Kanzei Chuo Bunsekishoho]* **1987**, *27*, 17-23.

Albach, R. F.; Redman, G. H.; Cruse, R. R. Annual and seasonal changes in naringin concentration of Ruby Red grapefruit juice. *J. Agric. Food Chem.* **1981a**, *29*, 808-811.

Albach, R. F.; Redman, G. H.; Cruse, R. R.; Petersen, H. D. Seasonal variation of bitterness components, pulp, and vitamin C in Texas commercial citrus juices. *J. Agric. Food Chem.* **1981b**, *29*, 805-808.

Arctander, S. *Perfume and flavor chemicals (Aroma chemicals)*; Vols. I and II; Allured Publishers: Carol Stream, IL, 1994.

Attaway, J. A. Factors influencing the flavor of grapefruit juice. *Proceedings of International Society of Citriculture*. **1977**, 816-820.

Attaway, J. A. Some new analytical indicators of processed orange juice quality. *Proc. Fla. State Horti. Soc.* **1972**, *85*, 192-203.

Barros, S. M.; Davis, J. E.; Dougherty, M. H.; Griffiths, J. T. Interrelationships of Brix, Brix-acid ratio, Naringin and Limonin and their effect on flavor of commercial, canned, single strength grapefruit juice. *Proc. Fla. State Horti. Soc.* **1983**, *96*, 316-318.

Bazemore, R.A. OSME and sensory analysis of aqueous orange essence; M.S. Thesis. Oregon State University. Corvallis, OR. 1995.

Berry, R. E.; Tatum, J. H. Bitterness and immature flavor in grapefruit analysis and improvement of quality. *J. Food Sci.* **1986**, *51*, 1368-1369.

Boelens, M. H.; Gemert, L. J. van. Volatile character-impact sulfur compounds and their sensory properties. *Perfumer & Flavorist.* **1993**, *18*, 19-34.

Burroughs, L.F. Amino acids. In *The biochemistry of fruits and their products*; Hulme, A.C., Ed.; Academic Press: New York, 1970; pp. 119-146.

Buttery, R.G.; Ling, L.C. Methods for isolating food and plant volatiles. In *Biotechnology for improved foods and flavors*, ACS Symposium Series, 1996; pp 240-247.

Cadwallader, K. R.; Xu, Y. Analysis of volatile components in fresh grapefruit juice by purge and trap/gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 316-318.

Chien, M.; Peppard, T. Use of statistical methods to better understand gas chromatographic data obtained from complex flavor systems. In *Flavor Measurement*; Ho, C.T., Manley, C.H., Eds.; Marcel Dekker, Inc: New York, 1992; pp 1-35.

Chin, H. W.; Bernhard, R. A.; Rosenberg, M. Solid phase micro extraction for cheese volatile compound analysis. *J. Food Sci.* **1996**, *61*, 1118-1122.

Citrus Summary, Florida Agricultural Statistics Service: Orlando, FL. 1996-97.

Clark, B.C.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-flavors in foods and beverages*; Charalambous, G., Ed.; Elsevier: New York, 1992; pp 229-286.

Cunnigham, D.G.; Acree, T.E.; Barnard, J. Charm® Analysis of Apple Volatiles. *Food Chem.* **1986**, *19*, 137-147.

da Silva, M.A.A.P.; Lundahl, D.S.; McDaniel M.R. The capabilities and psychophysics of Osme: a new GC-Olfactometry technique. In *Trends in flavor research*; Maarse, H., Van Der Heij, Eds.; Elsevier: New York, 1994; pp 191-210.

Demole, E. P.; Enggist, P. [Utilization of a sesquiterpene ketone as perfume/flavour compound.] *European Patent.* **1986**.

Demole, E.; Enggist, P.; Ohloff, G. 1-p-menthene-8-thiol: A powerful flavor impact constituent of grapefruit juice (*Citrus paradisi Macfadyen*). *Helvetica Chimica Acta.* **1982**, *65*, 1785-1794.

Fellers, P. J. Florida's citrus juice standards for grades and their differences from United States standards for grades and united states food and drug administration standards of identity. *Proc. Fla. State Hort. Soc.* **1990**, *103*, 260-265.

Fellers, P. J. The relationship between ratio of degrees Brix to percent acid and sensory flavor in grapefruit juice. *Food Technol.* **1991**, *45*, 68, 70, 72-75.

Fellers, P. J.; Carter, R. D.; Jager, G. de. Influence of limonin on consumer preference of processed grapefruit juice. *J. Food Sci.* **1987**, *52*, 741-743.

Fellers, P. J.; Jager, G. De.; Poole, M. J.; Hill, E. C.; Mittal, P. Quality of Florida-packed retail grapefruit juices as determined by consumer sensory panels and chemical and physical analyses. *J. Food Sci.* **1986**, *51*, 417-420.

Guadagni, D.G.; Maier, V.P.; Turnbaugh, J.G. Effect of some citrus juice constituents on taste thresholds of limonin and naringin bitterness. *J. Sci. Fd Agric.* **1973**, *24*, 1277-1288.

Hasegawa, S.; Pelton, V. A.; Bennett, R. D. Metabolism of limonoids by Arthrobacter globiformis II: Basis for a practical means of reducing the limonin content of orange juice by immobilized cells. *J. Agric. Food Chem.* **1983**, *31*, 1002-1004.

Hinterholzer, A; Schieberle, P. Identification of most odour active volatiles in fresh hand extracted juice of Valencia late oranges by odour dilution techniques. *Flavor and Fragrance Journal.* **1998**, *13*, 49-55.

Jella, P.; Rouseff, R.; Goodner, K.; Widmer, W. Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. *J. Agric. Food Chem.* **1998**, *46*, 242-247.

Jennings, W. G. Objective measurements of flavor quality: general approaches, problems, pitfalls, and accomplishments. *ACS. Symp. Ser.* **1977**, *51*, 1-10.

Johnson, R. L.; Chandler, B. V. Ion exchange and adsorbent resins for removal of acids and bitter principles from citrus juices. *J. Sci. Food Agric.* **1985**, *36*, 480-484.

Kimball, D.A. Debittering of citrus juices using supercritical carbon dioxide. *J. Food Sci.* **1981**, *52*, 481-482.

Kimball, D.A. *Citrus processing - quality control and technology*; Van Nostrand Reinhold: New York, 1991.

Klim, M.; Nagy, S. Analysis of orange juice volatile: Comparison of extraction with Freon 113 and ethyl acetate. *Proc. Fla. State Hort. Soc.* **1992**, *105*, 110-112.

Kovats, E. *Advances in Chromatography*, vol. 1. Giddings J.C., Keller R.A., Eds.; Marcel Dekker: New York. 1965; 229-247.

Maarse, H.; Visscher, C.A. *Volatile compounds in Food-qualitative and quantitative data*, vol. 1, TNO-Civo Food Analysis Institute: The Netherlands, 1989.

Marcotte, M.; Stewart, B.; Fustier, P. Abused thermal treatment impact on degradation products of chilled pasteurized orange juice. *J. Agric. Food Chem.* **1998**, *46*, 1991-1996.

Marin, A. B.; Acree, T. E.; Hotchkiss, J. H.; Nagy, S. Gas chromatography-olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* **1992**, *40*: 650-654.

Matich, A. J.; Rowan, D. D.; Banks, N. H. Solid phase micro extraction for quantitative headspace sampling of apple volatiles. *Analytical Chemistry* **1996**, *68*, 4114-4118.

Meilgaard, M.; Civille, G.V.; Carr, B.T. *Sensory Evaluation Techniques.* CRC Press, Inc.: Boca Raton, FL, 1991; pp 49-50.

Miller, J.M.; Rockland, L.B. Determination of cysteine and glutathione in citrus juices by filter paper chromatography. *Archs. Biochem. Biophys.* **1952**, *40*, 416-423.

Miranda-Lopez R.; McDaniel, M.R.; Watson, B.T.; Libbey, L.M. Odor analysis of Pinot Noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (OSME). *J. Food Sci.* **1992**, *57*, 985-993.

Mistry, B.S.; Reineccius, G.A.; Jasper, B.L. Comparison of gas chromatographic detectors for the analysis of volatile sulfur compounds in foods. In *Sulfur compounds in Foods*; Mussinan, C.J., Keelan, M.E., Eds.; ACS Symposium Series-564: 1994; pp 8-21.

Mistry, B.S.; Reineccius, T.; Olson, L.K. Gas chromatography-olfactometry for the determination of key odorants in foods. In *Techniques for analyzing food aroma*; Marsili R., Ed.; Marcel Dekker: New York, 1997; pp 265-292.

Moshonas, M. G.; Shaw, P. E. Analysis of volatile flavor constituents from grapefruit essence. *J. Agric. Food Chem.* **1971**, *19*, 119-120.

Moshonas, M. G.; Shaw, P. E. Irradiation and fumigation effects on flavor, aroma and composition of grapefruit products. *J. Food Sci.* **1982**, *47*, 958-960.

Moshonas, M.G.; Shaw, P.E. Comparison of Static and Dynamic Headspace Gas Chromatography for Quantitative Determination of Volatile Orange Juice Constituents. *Food Science and Technology - Lebensmittel - Wissenschaft & Technologie.* **1992**, *25*, 236-239.

Núñez, A. J.; Bemelmans, J. M. H.; Maarse, H. [Isolation methods for the volatile components of grapefruit juice. Distillation and solvent extraction methods.]. *Chromatographia* **1984**, *18*, 153-158.

Núñez, A. J.; Maarse, H.; Bemelmans, M. H. Volatile flavor components of grapefruit juice (Citrus paradisi Macfadyen). *J. Sci. Food. Agric.* **1985**, *36*, 757-763.

Ohloff, G. The chemical senses. In *Scent and Fragrances;* Springer-Verlag: New York, 1990; pp 1-9.

Parliament, T. H. A new technique for GLC sample preparation using a novel extraction device. *Perfumer and Flavorist.* **1986**, *11*, 1-8.

Peleg, H.; Naim, M.; Zehavi, U.; Rouseff, R. L.; Nagy, S. Pathways of 4-vinylguaiacol formation from ferulic acid in model solutions of orange juice. *J. Agric. Food Chem.* **1992**, *40*, 764-767.

Pelusio, F.; Nilsson, T.; Montanarella, L.; Tilio, R.; Larsen, B.; Facchetti, S.; Madsen, J. O. Headspace solid-phase micro extraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J. Agric. Food Chem.* **1995**, *43*, 2138-2143.

Pino, J. A. Correlation between sensory and gas chromatographic measurements on orange volatiles. *Acta Alimentaria.* **1982**, *11*, 1-9.

Pino, J. A.; Cabrera, M. Quality of canned grapefruit juice produced in Cuba for four seasons. *Nahrung.* **1988**, *32*, 875-879.

Pino, J. A.; Torricella, R.; Orsi, F. Correlation between sensory and gas chromatographic measurements on grapefruit juice volatiles. *Acta Alimentaria*. **1986a**, *15*, 237-246.

Pino, J. A.; Torricella, R.; Orsi, F.; Figueras, L. Application of multivariate statistics for the quality classification of single-strength grapefruit juice. *J. Food Qual.* **1986b**, *9*, 205-216.

Reneccius, G.A. *Source book of flavors - 2nd edition*; Chapman and Hall: New York, 1994.

Rouseff, R. L. Nomilin a new bitter compound in grapefruit juice. *J. Agric. Food Chem.* **1982**, *30*, 504-507.

Rouseff, R. L.; Barros, S. M.; Dougherty, M. H.; Martin, S. F. A survey of quality factors found in Florida canned single strength grapefruit juice from 1977-78, 1978-79, and 1979-80 seasons. *Proc. Fla. State Hort. Soc.* **1980**, *93*, 286-289.

Rouseff, R.; Nagy, S. A multivariate pattern recognition approach for identifying quality factors in Florida orange juice. In *Flavor Science and Technology*. Martens, M., Dalen G.A., Russwurm, H., Jr. Eds. Wiley: New York, 1982.

Schieberle, P.; Grosch. W. Identificierung von Aromastoffen ausder Kruste von Roggenbrot - Verleich mit den Aromastoffen der Krume. *Z. Lebensm. Unters. Forsch.* **1984**, 479-483.

Schieberle, P.; Grosch. W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.

Shaw, P.E. Fruits II. In *Volatile compounds in foods and beverages*; Maarse, H., Ed.; Marcel Dekker: New York, 1991.

Shaw, P.E. Volatile components important to citrus flavors. In *Quality control manual for citrus processing plants*; Red, J.B., Shaw, P.E., Hendricks, C.M., Hendricks, D.L.: Eds.; Ag. Science: Florida, 1996, 134-172.

Shaw, P. E.; Ammons, J. M.; Braman, R. S. Volatile sulfur compounds in fresh orange and grapefruit juices: identification, quantitation, and possible importance to juice flavor. *J. Agric. Food Chem.* **1980**, *28*, 778-781.

Shaw, P.E.; Nagy, S. Analysis and flavor effects of volatile sulfur compounds in citrus juices. In *The quality of foods and beverages*; Academic Press: New York, 1981; pp. 361-376.

Shaw, P. E.; Wilson, C. W, III. Importance of nootkatone to the aroma of grapefruit oil and the flavor of grapefruit juice. *J. Agric. Food Chem.* **1981**, *29*, 677-679.

Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase micro extraction. *J. Agric. Food Chem.* **1996**, *44*, 2187-2193.

Stevens, K. L.; Guadgni, D. C.; Stern, D. J. Odour character and threshold values of nootkatone and related compounds. *J. Agric. Food Chem.* **1970**, *21*, 590-593.

Stevenson, R. J.; Chen, X. D. Solid-phase micro extraction: a useful sample preparation technique for gas chromatography-mass spectrometry analysis of volatile components in food products. *Food Technol.* **1996**, *26*, 24-28.

Stinson, W.S.; Barros, S. Grapefruit juice improvement program. *Proceedings of citrus short course meeting*, Clearwater, FL. 1997, pp 113-126.

Tatum, J. H.; Lastinger, Jc, Jr.; Berry, R. E. Naringin isomers and limonin in canned Florida grapefruit juice. *Proceedings of the Florida State Horticultural Society.* **1972**, *85*, 210-213.

Tressl, R.; Silwar, R. Investigation of sulfur-containing components in roasted coffee. *J. Agric. Food Chem.* **1981**, *29*, 1078-1082.

Umano, K.; Shibamoto T Aia: United States of America, F. M. A. A new method of headspace sampling: grapefruit volatiles. (In `Flavors and fragrances: a world perspective', edited by B. M. Lawrence et al. Conference. Washington, DC, USA. 16-20 Nov. 1986. Amsterdam, Netherlands; Elsevier Science Publishers BV. 1SBN 0-444-42964-6.). *Developments in Food Science* **1988**, *18*, 981-998.

Velez, C.; Costell, E.; Orlando, L.; Nadal, M. I.; Sendra, J. M.; Izquierdo, L. Multidimensional scaling as a method to correlate sensory and instrumental data of orange juice aromas. *J. Sci. Food Agric.* **1993**, *61*, 41-46.

Wampler, T.P. Analysis of food volatiles using headspace-gas chromatographic techniques. In *Techniques for analyzing food aroma*; Marsili R., Ed.; Marcel Dekker: New York, 1997; pp 27-58.

Weurman C. Isolation and concentration of volatiles in food odor research. *J. Agric. Food Chem.* **1969**, *17*, 370-384.

Widmer, W. W.; Martin, S. F. Analysis of limonin in citrus juices by direct injection and online sample clean up. *Abstracts of Citrus Processors Meeting*, October, 1994, Lake Alfred, FL, 1994.

Wilson, C. W.; Wagner, C. J.; Shaw, P. E. Reduction of bitter components in grapefruit and navel orange juices with b-cyclodextrin polymers or XAD resins in a fluidized bed process. J. Agric. Food Chem. **1989**, *37*: 14-18.

Xiaogen, Y.; Peppard, T. Solid-phase micro extraction for flavor analysis. *Journal of Agricultural and Food Chemistry* **1994**, *42*, 1925-1930.

Zhang, Z.; Yang, M.J.; Pawliszyn, J. Solid-phase micro extraction. *Anal. Chem.* **1994**, *66*, 844-853.

BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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